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Gimme shelter

Combining free-range broiler chickens with production of short rotation coppice

Thesis submitted in fulfilment of the requirements
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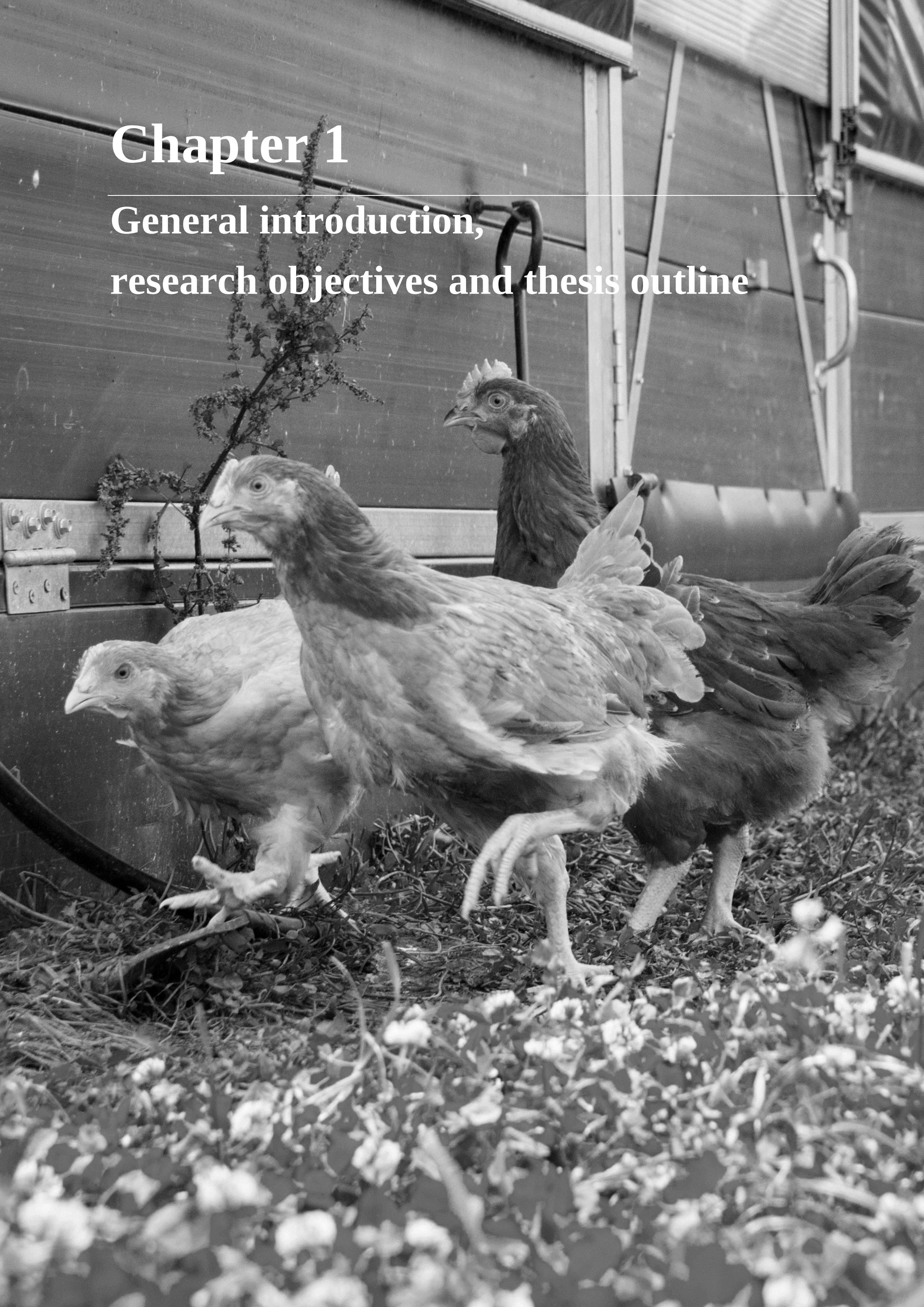
List of abbreviations

Shelter types	
AS	Artificial shelter
IN	Indoors
SRCW	Short rotation coppice willow
Weather conditions	
DPT	Dew point temperature
HI	Heat index
RH	Relative humidity
WC	Wind chill
Leg health	
FPD	Foot pad dermatitis
Behavioural tests	
OF	Open field
TI	Tonic immobility
Meat quality and production	
ADG	Average daily gain
BW	Body weight
FCR	Feed conversion ratio
FI	Feed intake
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
WHC	Water-holding capacity
Positioning system	
APS	Automated positioning system
GPS	Global positioning system
RFID	Radio-frequency identification
UWB	Ultra-Wideband
Nutrients	
AL	Ammonium lactate
C	Carbon
Ca	Calcium
Cl	Chloride

Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NH_3	Ammonia
NH_4-N	Ammonia nitrogen
N_{min}	Mineral nitrogen
NO_3-N	Nitrate nitrogen
N_{tot}	Total nitrogen
P	Phosphorus
TOC	Total organic carbon
Statistics	
CI	Confidence interval
DFFITS	Difference in fits
LS means	Least squares means
Other	
AI	Avian influenza
DM	Dry matter

Chapter 1

General introduction,
research objectives and thesis outline



1.1 Free-range chicken production in Belgium: market and regulations

In Western countries, the majority of broiler chickens (i.e. chickens for meat production) are housed indoors in intensive systems. However, a smaller share is housed with access to a free-range area, and in some countries this share is increasing. Of all broiler chickens in Belgium, ca. 1.3 – 2% is raised in organic production systems, so with outdoor access (BioWallonie, 2015; Samborski and Van Bellegem, 2016; VLAM, 2015). Reliable statistics about free-range broiler chickens (i.e. not organic, but with outdoor access) are lacking, but it is estimated that 14,000 free-range broilers are slaughtered on a weekly basis in Belgium (Bergen, 2015), which corresponds to 0.3% of all broiler chickens slaughtered (FOD Economie, 2016). So, it can be estimated that ca. 1.6 – 2.3% of all broiler chickens in Belgium have free-range access.

The market share of organic chicken meat in Belgium is growing (Samborski and Van Bellegem, 2016), and a recent study showed that Belgian consumers are willing to pay more for meat from chickens reared in free-range systems (Van Loo et al., 2014). Housing systems with outdoor access are generally perceived by consumers as more natural, and beneficial for animal welfare (de Jonge and van Trijp, 2013; Vanhonacker et al., 2016, 2008). This could contribute to consumers' decision to buy free-range or organic products (Vanhonacker et al., 2010; Vanhonacker and Verbeke, 2009). Other reasons for increased willingness to pay may include consumers' perception that free-range or organic meat is healthier or tastes better (Vanhonacker and Verbeke, 2009).

Meat from organic poultry can be recognised by the European organic label. Meat from free-range broiler chickens is only allowed to be sold as "free range" when the production complies with EU regulations on outdoor access (European Commission, 2008a); however, there is no clear labelling system for free-range poultry meat from Belgium. A well-known brand of free-range chicken meat sold in Belgium is Label Rouge. Farms producing these chickens have to comply with Label Rouge standards, which include free-range access. However, these chickens are all imported from France, and no similar production chain and branding exists in Belgium yet.

Organic broiler chickens are required to have outdoor access for at least one third of their lives (European Commission, 2008b); in Belgium they are usually kept indoors during the first 5 weeks of their lives, after which they get access to 4 m^2 of outdoor space per bird, until they reach slaughter age (usually at 10 weeks; Table 1.1). If poultry meat is labelled "free range",

Table 1.1 Overview of requirements regarding stocking density, outdoor access, slaughter age, genetic strain and flock size for organic and free-range broiler production systems.

Production system	Maximum indoor stocking density (birds/m²)	Minimum outdoor space per bird (m²)	Minimum period of outdoor access	Minimum slaughter age (d)	Breeds	Maximum flock size
Organic	10 (max 21 kg live weight)	4	A third of the birds' life	70 (slow-growing strains) 81 (other strains)	Slow-growing strains[1]	4 800
Free range	13 (max 27.5 kg live weight)	1	Half the birds' life	56	Not specified	Not specified
Traditional free range	12 (max 25 kg live weight)	2	From 6 weeks of age onwards	81	Slow-growing strains[1]	4 800
Free range – total freedom	12 (max 25 kg live weight)	Unlimited	From 6 weeks of age onwards	81	Slow-growing strains[1]	4 800

[1] *Wallonia: Sasso XL451; Flanders: Sasso XL451, Sasso X451, Hubbard JA57 x I66C, Kabir 277 x GGKNN, Kabir 277 x GGK, Kabir 99 x GGKNN.*

chickens should have had at least 1 m^2 outdoor space per bird, 2 m^2 if it is labelled "traditional free range" or unlimited outdoor area if labelled "free range – total freedom" (European Commission, 2008a; Table 1.1). The Label Rouge is an example of "traditional free range", with 2 m^2 outdoor space per chicken.

For organic broiler production in Belgium, slow-growing breeds are required. In practice, the most common strain in Belgium is the Sasso XL451. In Wallonia, where 90% of Belgian organic-broiler production is situated (Stefan D'Hulster, Bio'Or, personal communication), only this strain is allowed to be slaughtered for organic production at 70 days of age (for other strains a minimum age of 81 days is required; Verbeke, 2012b), while in Flanders five other strains are also allowed to be slaughtered at 70 days (Table 1.1; Vlaamse Overheid, 2009). The Sasso XL451 are robust chickens, bred specifically for organic production. They are slow-growing with an average daily gain of ca. 40 g as compared to ca. 65 g for Ross 308 chickens, which is a conventional fast-growing strain. In practice they are slaughtered at 70 days of age, instead of at 42 days for Ross 308. If fast-growing chickens would be used for organic production, they would have to be reared until day 81, at which point they would weigh around

3.5 – 4.0 kg leading to poor leg health and high mortality and therefore impaired welfare (Castellini et al., 2002a; Weeks et al., 1994). Additionally, the high weight can be problematic for the slaughterhouse because the size of the equipment is unsuitable and the products are difficult to market (Dirk Cools, Belki, personal communication).

Thesis focus 1: Broiler chickens

In this thesis, the focus will be on broiler chickens with free-range access. The reasons to focus on broiler chickens and not on laying hens are twofold. Firstly, free-range use is often poorer in broiler chickens than in laying hens so there is more potential for improvement. Secondly, research on broiler chickens with free-range access is more scarce than in laying hens, and it is not certain if results from studies with laying hens can be extrapolated to broilers. Given the limited amount of research on free-range broiler chickens available, research on laying hens with free-range access will also be taken into account when reviewing existing literature. Although laying hens differ from broiler chickens in age and physique, both descend from the jungle fowl and have a behavioural repertoire that is at least partially similar (Duncan, 1998). Therefore, results found in studies with laying hens may give indications for promising research directions in broiler chickens and in turn, studies on broilers, including the current study, may be relevant to laying hen production.

1.2 Potential effects of free-range access on the animals and the environment

Free-range housing systems are vastly different from conventional, indoor systems: e.g. the birds have more space, environmental diversity is higher, the diet is different (access to plant material and insects), birds may be exposed more to environmental risks such as infectious diseases and the birds could impact the environment through emissions and faeces deposition. This means that free-range access affects multiple parameters such as animal welfare and behaviour, production and meat quality and the environment. These will be discussed in more detail below.

1.2.1 Animal welfare and behaviour

Animal welfare can be defined in several ways. The first widely-used definition described animal welfare using the five freedoms (freedom from hunger and thirst, discomfort, disease and pain, fear and distress, and free to perform natural behaviours; FAWC, 1979). Later, welfare has been defined as “the state of an individual in relation to its environment”, where "both failure to cope with the environment and difficulty in coping are indicators of poor welfare" (Broom, 1991). Three views emerged, one focussing on natural living, one on good biological functioning, and one on a positive emotional state (Duncan and Fraser, 1997). Ultimately, welfare will be defined by the animal's emotional state (Duncan, 2002) and not by good biological functioning or natural living, although these may contribute to the emotional state. Part of natural living is the ability to perform natural, highly motivated behaviours. Behavioural needs are behaviours that are controlled largely by internal factors (Duncan, 1998), and if animals are unable to adequately perform them this may lead to suffering (Jensen and Toates, 1993). Example of such behaviours in chickens are foraging and nest-building (Weeks and Nicol, 2006). The following paragraphs will discuss several factors that may differ between birds with and without outdoor access, and could have an impact on their welfare. It has to be noted that effects on broiler welfare that can be attributed solely to outdoor access are difficult to identify because these are often confounded with effects of many other factors that differ between indoor and free-range production systems, such as the use of slower-growing genotypes, more space per animal, and different diets (Tuyttens et al., 2008). In addition, the effects of free-range access can vary depending on the extent to which the range is used by the chickens.

Leg health Access to a range can be perceived as a form of environmental enrichment, and enrichment has the potential to positively affect broiler chickens' behaviour, e.g. resulting in higher activity levels (Kells et al., 2001; Nicol, 1992), which could subsequently promote better leg health (Bizeray et al., 2002; Leterrier et al., 2008). Impaired leg health could cause pain, but also stress or fear resulting from a reduced capability to perform certain behaviours or flee from aggressive conspecifics. Although causality could not be established, Jones et al. (2007) found that broiler chickens that ranged more had better gait scores and less foot pad dermatitis (**FPD**). Broilers typically are more active and exploratory outside, while they rest more inside the house (Fanatico et al., 2016; Taylor et al., 2015). In addition to range access, shelter or enrichment on the range also have effects on behaviour and welfare. Broilers with sorghum or olive trees on the range spent less time lying and more time moving, and had lower incidences of FPD and breast blisters than those with a barren range (Dal Bosco et al., 2014). Broilers with perches or vertical shelters on the range were less likely to be observed sitting than birds without enrichment (Fanatico et al., 2016). Shelterbelts (rows of trees) led to more foraging and running in laying hens (Borland et al., 2010). More insight in the beneficial effects of free-range use and how shelter could aid in this would help to further promote animal welfare in free-range systems.

Results from studies regarding FPD in broilers in relation to organic production systems are conflicting, with some studies finding higher prevalences of (severe) FPD in organic systems compared to conventional (Pagazaurtundua and Warriss, 2006), and others lower (Bokkers and de Boer, 2009). Litter quality may be poorer in free-range systems due to muddy conditions of the range surrounding the house. On the other hand, higher activity levels of the birds and more ventilation could improve litter quality (Bailie et al., 2013; Weaver and Meijerhof, 1991). Another risk factor for FPD is high slaughter age (Kyvsgaard et al., 2013), which occurs in free-range broiler production. The reason for this may be the prolonged contact of the feet with the litter, and the poorer feed conversion ratio (**FCR**) leading to more nitrogen **(N**) losses with the droppings (Kyvsgaard et al., 2013). A measure that may indicate wet litter and thus a risk for dermatitis (de Jong et al., 2016; Shao et al., 2015) is soiled plumage. It might also indicate a lower activity level, which could be related to (leg) health. In addition, soiled plumage can lead to impaired thermoregulation (Welfare Quality®, 2009).

Health For animal and public health, outdoor access for broilers may pose a risk with regards to *Campylobacter* infections (Näther et al., 2009), although not all studies found this to be higher in free-range flocks (Economou et al., 2015; Tuyttens et al., 2008) or merely found higher prevalences of strains which are only responsible for a small minority of the *Campylobacter* outbreaks in humans (Rodenburg et al., 2004). Respiratory health status of the chickens has been demonstrated to be better in organic broiler flocks compared to conventional ones (Van Overbeke et al., 2006). Better air quality outside compared to in the chicken house could play a role in this.

It is sometimes assumed that outdoor access is a risk factor for infection with the avian influenza (**AI**) virus. Some studies found no evidence for this on different types of poultry farms (Snow et al., 2007; Thomas et al., 2005), but others found that outdoor layer farms have an increased risk of a factor between 6.3 – 11 relative to the risk in indoor farms' to be infected with AI (Bouwstra et al., 2017; Gonzales et al., 2013). This would be due to contact between domestic poultry and migratory birds, mainly water fowl, infected with the virus. One of the possible solutions for this could be planting trees on the range, because these may prevent water fowl from landing in the range (Bestman et al., 2017).

Studies on mortality in organic and conventional broiler farms show conflicting results (Bogosavljevic-Boskovic et al., 2012; Bokkers and de Boer, 2009). Again, besides free-range access, other factors associated with organic farming, such as the use of slow-growing strains, and farm-specific factors will play a role in these findings (Fanatico et al., 2008). One factor that can be responsible for mortality and which particularly occurs in free-range systems, is predation by e.g. foxes or raptors such as buzzards. The extent to which predation is a problem seems to be highly dependent on specific areas (Knierim, 2006). Suitable vegetation may prevent predation by raptors (Dal Bosco et al., 2014).

Natural behaviours and freedom of choice Animal welfare is generally perceived to be better in free-range systems than in indoor systems by consumers, because the environment is better suited to perform natural behaviours. In general, a range provides a higher quantity and quality of stimuli than an indoor environment, and will stimulate and facilitate exploratory and highly motivated foraging and dust bathing behaviours (Knierim, 2006).

It has been recognised by animal scientists that natural environments, such as outdoor access, generally provide animals with more freedom and opportunities to express natural behaviours (Miele et al., 2011). They can choose if they want to be indoors or outdoors, depending on e.g. weather conditions, social motivation, hunger or thirst, or foraging motivation. This controllability can improve their welfare by reducing stress levels (Wiepkema and Koolhaas, 1993). In addition, outdoor access provides birds with the opportunity to partially select their own diet through the ingestion of e.g. plant material, worms, and insects. Previous studies showed that broiler chickens are capable of selecting diets which are best for their performance (Erener et al., 2003; Gous and Swatson, 2000), and a range with sufficient substrates for foraging could facilitate this behaviour.

1.2.2 Production and meat quality

Free-range access may influence production parameters in broiler chickens, such as average daily gain (**ADG**) and meat quality. Because it is likely that free-range access results in more activity, the birds may have a poorer FCR, sometimes resulting in lower ADG compared to birds kept indoors (Castellini et al., 2002b; Wang et al., 2009). However, other studies found no difference (Fanatico et al., 2005a; Tong et al., 2014) or even a higher ADG in chickens with outdoor access (Ponte et al., 2008b, 2008c). Further studies are necessary in order to identify the factors responsible for these differences. One factor which may be responsible is the difference in free-range use between the studies; if free-range use is low it may be less likely to find weight differences between indoor and outdoor groups. One study comparing groups of chickens with different periods of outdoor access found a higher ADG in chickens with more days of outdoor access (Tong et al., 2015), although two other studies found no effect of free-range use (expressed as the average percentage of animals observed outside) on ADG (Dal Bosco et al., 2014; Dawkins et al., 2003).

Quality of the meat may be affected by free-range access through more exercise (alterations to muscle fibre characteristics) and a different diet (intake of plants, worms and insects). Exercise can cause a shift in muscle fibres from type IIB (white, anaerobic) to type I (red, aerobic) especially in leg muscles (Branciari et al., 2009). It can also lead to an increase in the total number of fibres, or in the fibres' diameter (Brackenbury and Holloway, 1991; Giddings and Gonyea, 1992; Gonyea et al., 1986). Colour of the meat of free-range chickens has been found to be more yellow than that of indoor birds, probably due to higher levels of carotenoid intake (Castellini et al., 2002b; Fanatico et al., 2007; Sales, 2014). However, research on other parameters such as tenderness, water-holding capacity (**WHC**), and protein content shows conflicting results, and it is unclear why this inconsistency exists (Castellini et al., 2002b; Chen et al., 2013; Jiang et al., 2011; Mikulski et al., 2011; Sun et al., 2013; Wang et al., 2009). Again, this may be due to differences in free-range use between the studies. In one study the breast yield increased and the meat was yellower with increasing days of outdoor access (Tong et al., 2015), indicating that the extent of free-range use indeed has an effect on these parameters. Unfortunately, most studies concerning meat quality did not quantify free-range use. Including this could shed more light on the relationship between these parameters.

1.2.3 Vegetation, environment and ecosystem services

If chickens are provided with outdoor access, there are direct effects they can have on the environment, such as point pollution, but also emissions (NH_3 and particulate matter). In addition, there are interactions between the birds, the vegetation and the environment. For example, a high density of chickens may lead to point pollution with high levels of N and phosphorus (**P**), but this may be mitigated by vegetation capable of taking up these nutrients from the soil. These aspects will be discussed in the following paragraphs.

Point pollution In addition to affecting animal-related variables, free-range access also influences the environment, including the soil and the vegetation in the range. High concentrations of chickens can often be found close to the chicken house. As a result, high levels (exceeding threshold values) of N have been found close to laying hen and broiler houses (Aarnink et al., 2006; Dekker et al., 2012; Elbe et al., 2005; Kratz et al., 2004). In addition, levels of P excretion and soil P were higher on plots that were used most by free-range broiler chickens (Kratz et al., 2004; Méda et al., 2015). This poses a risk for leaching of N and P to the groundwater, which could lead to eutrophication and subsequently increased algae growth, hypoxia and reduced aquatic biodiversity. A better distribution of the chickens, and thus their faeces, over the entire range may diminish this risk. However, more chickens going outside may again increase the risk of too high concentrations of these nutrients. Therefore, it is important to monitor how free-range use influences these parameters.

It is important to note that no matter how heterogeneously the range is used, total N and P excretions will remain the same. Where they are deposited, however, is of great importance for the chickens, the farmer and the environment. Studies with laying hens showed that a high concentration of chickens close to the house leads to deposition of faeces in this area, but also to depletion of vegetation (Aarnink et al., 2006; Bubier, 1998) that could potentially take up these nutrients so they would not leach to the groundwater. In addition, the high concentration of faeces might cause odour nuisance for neighbouring residents or increased risk of infectious diseases for the chickens. It is practically difficult to collect the faeces from the range so that they can be recycled as fertiliser. Therefore, a good distribution of the flock over the range is important so that the deposited nutrients can be used by the vegetation.

Minimising point pollution can also be achieved by using mobile chicken houses. These are units capable of housing up to several thousand chickens, which can be moved to different locations on the range. They have to be moved to a different plot of land at least once per year (Vlaamse Overheid, 2011). Thereby, the areas with high faeces concentration (i.e. close to the house) change location, thus decreasing the risk of high concentrations of e.g. P and N, and preventing leaching of those nutrients to groundwater. The vegetation which is depleted close to the house also gets time to recover when the position of the house is rotated. Another advantage of using mobile houses is that for broiler chickens, the maximum indoor and outdoor stocking density can be increased; the indoor stocking density can be raised from 10 to 16 birds per m^2, and only 2.5 m^2 instead of 4 m^2 outdoor space per chicken is required (European Commission, 2008).

A high concentration of chickens close to the house can lead to point pollution but also to soil compaction (Castellini et al., 2012; Clark and Gage, 1997), causing puddles of water to persist on the range. Broiler chickens have been observed to drink from these puddles (Bogosavljevic-Boskovic et al., 2012; Jones et al., 2007), which in turn could lead to the spread of infectious diseases (Humphrey et al., 2005; Johnsen et al., 2006; Ring et al., 2005). It may also result in poorer litter quality as chickens will walk into the house with wet, muddy feet. Soil compaction also means there is less water and nutrients in the soil, leading to reduced plant growth (Hamza and Anderson, 2005). Again, a better distribution of the chickens over the entire range could diminish these problems.

Emissions In addition to point pollution, another disadvantage of free-range chicken production could be the increased emissions of ammonia (**NH_3**) and particulate matter as compared to indoor housing both in laying hens and broiler chickens (Bokkers and de Boer, 2009; Demmers et al., 2010). Vegetative shelterbelts may have the ability to trap NH_3 and dust, thus have the potential to mitigate this problem (Adrizal et al., 2008; Patterson and Adrizal, 2005). However, so far not many studies have yet been performed on this topic, and the few available studies often had conflicting results (Pronk et al., 2013). It seems that a reduction of particulate matter is more feasible than reductions of NH_3 and odour, and that the effects strongly depend on the configuration of the barn and the vegetation (Pronk et al., 2013).

Soil-vegetation-chicken interactions Soil parameters may not only be influenced by presence of chickens, but also by vegetation type or an interaction between the two. Trees may be capable of taking up nutrients from deeper soil layers than grass, and thereby influence the soil nutrient profile. Grassland, on the other hand, may take up nutrients from the top layer which are removed from the field if the grass is mown, while in trees the nutrients accumulate and will partially return to the soil through leaf fall. Jones et al. (2007) found no effect of tree presence on nitrate levels in the groundwater. Another study found increased levels of N leaching after converting grassland to short rotation coppice willows (**SRCW**; see 1.1.3 and Thesis focus 2), but this was only assessed shortly after conversion, and was not expected to last long-term due to increasing N demands of the trees (Nikièma et al., 2012). This is in accordance with findings that N concentrations in drainage water were high after initial establishment of the coppice, but decreased again subsequently (Goodlass et al., 2007). Another study found lower levels of N in groundwater in old (>10 years) SRCW plantations than on grassland even if fertiliser was applied to SRCW but not to grassland (Dimitriou et al., 2012a). In addition to influencing N levels, SRCW is perhaps more capable than grass of carbon (**C**) sequestration (Dimitriou et al., 2012b; Grogan and Matthews, 2002), which is the process of storing atmospheric carbon dioxide in the soil and therefore relevant for the deceleration of global warming.

Vegetation on the range may also be affected by the presence of chickens and the extent of their range use. The chickens could have positive effects such as fertilisation of the soil and controlling weed growth and pest insects, but may also cause damage to the grass and (young) trees by eating the fresh leaves or exposing the roots of the trees through foraging behaviour. However, few studies have assessed this. One study found that, on plots where broilers were present, broadleaf and conifer trees were somewhat smaller compared to plots without chickens, but this was measured two years after trees were planted, and no conclusions on long-term effects could be drawn (Jones et al., 2007). Experience from practice indicates that it is necessary to fence off newly planted SRCW from laying hens until the trees are 2.5 – 3 months old in order to prevent damage (Boosten, 2015).

1.2.4 Conclusion

Overall, the effects of providing broiler chickens with free-range access can be both positive and negative. For animal welfare the balance seems to be positive, with more possibilities to perform natural behaviours, more freedom of choice and possibly better leg health. For meat quality, the effects are less clear. There are, however, indications that free-range access most certainly has an effect, and perhaps better quantification of free-range use may give more insights in the relationships. From the environmental point of view, free-range access brings several challenges such as point pollution, damage to vegetation and emissions. Solutions for these issues such as better distribution of the flock over the range, use of mobile houses, using vegetation to take up nutrients need to be further investigated.

1.3 Factors influencing free-range use

A problem with free-range chicken production is that usually only a minority of the flock is outside. Previous studies with broiler chickens found that between 5 and 13% of the birds were outside at any given moment during the period the pop holes were open (Dawkins et al., 2003; Fanatico et al., 2016; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). In addition, those that do go outside often stay close to the house (Dawkins et al., 2003; Fanatico et al., 2016; Taylor et al., 2015). A better distribution of the flock over the entire free-range area would minimise point pollution, and lead to a lower local stocking density. Moreover, using the entire range could benefit chicken welfare because they would have access to more substrates for foraging and dust bathing, and more space per animal.

Broiler chicken farmers perceive outdoor access to be harmful for animal welfare, because it can increase the risk for diseases (Tuyttens et al., 2014). In addition they perceive outdoor access to have a negative effect on product safety and work load (Tuyttens et al., 2014). Consumers, on the other hand, do associate outdoor access with better animal welfare (de Jonge and van Trijp, 2014, 2013, Vanhonacker et al., 2016, 2008), and state in surveys to be willing to pay a price premium for free-range broiler meat as compared to conventional meat (Van Loo et al., 2014). The difference in perception may be attributed to the higher importance given to natural living by consumers than by broiler farmers (Howell et al., 2016). However, if free-range use is low, it can be questioned how beneficial such a system is for animal welfare, and whether it is fair to market the meat from these systems as ‘free-range’. Therefore, it is important to search for strategies to improve free-range use so that the perceived better welfare status (by consumers) can be achieved, while also monitoring possible negative effects on chicken health.

It is still unclear why the free-range use of broiler chickens is so limited. How well an animal ‘fits’ in its environment depends both on the environment and on the animal (Lawrence and Wall, 2014; Phocas et al., 2016; Star et al., 2008). Therefore, when there is a mismatch between the animal and the environment, as seems to be the case for free-range chickens when range use is low, solutions can be sought in both the environment and the animal. Alterations to the environment could comprise provision of suitable shelter against (perceived) threats and protection from adverse weather conditions. Alterations to the animal may not exclusively comprise alterations to the genotype but also altering animal characteristics such as fearfulness by applying a more appropriate rearing strategy. Several aspects that are related with free-range are discussed in more detail below.

1.3.1 Shelter

Unsuitable shelter on the range might be a reason for suboptimal free-range use. Domestic chickens originate from the jungle fowl, which live in habitats with dense vegetation (Collias and Collias, 1967). In contrast, most broiler chicken ranges are covered mainly with grass without much shelter (Stefan D’Hulster, Bio’Or, personal communication). This makes the birds vulnerable for attacks from predators, especially raptors, and for adverse weather conditions. EU legislation prescribes that free-range areas for organic broiler chickens should be “mainly covered with vegetation and be provided with protective facilities” (European Commission, 2008b), but for free-range, non-organic, chickens legislation only states the range should be “mainly covered by vegetation” (European Commission, 2008a). However, neither the number, characteristics or total area of protective facilities nor the type of vegetation are specified, so vegetation can also be grassland.

Empirical evidence exists showing that broiler chickens, like their ancestors, prefer shelter over open areas. However, it is not yet clear which shelter types, or what characteristics of shelter are successful in promoting free-range use. Both artificial shelters such as vertical screens, overhead shade panels or camouflage nets (Fanatico et al., 2016; Rivera-Ferre et al., 2007b; Stadig et al., 2014; Zeltner and Hirt, 2003) and natural shelter such as olive trees, sorghum, or shelterbelts (Borland et al., 2010; Dal Bosco et al., 2014; Dawkins et al., 2003) appear to promote free-range use. However, natural shelters generally had a larger positive impact on free-range use in laying hens (Gilani et al., 2014; Nagle and Glatz, 2012). This may be related to that it may be easier to create large sheltered, shaded areas with trees than with e.g. wooden panels, that the microclimate is better under the natural than under the artificial shelter, or that natural shelter provides other benefits besides shelter, such as higher numbers of e.g. insects and more vegetation to forage on.

The optimal design of natural shelter remains to be studied. Projects in which fruit trees, Miscanthus, willows, or broad leaf trees and conifers have been planted in free-range areas all showed beneficial effects on free-range use by laying hens and broilers (Bestman, 2015; Jones et al., 2007). However, factors that are likely to impact free-range use are e.g. the type of vegetation, the amount of range that is covered, or the way the shelter is distributed over the range. Bright et al. (2011, 2016) showed that not the amount of range that was covered with vegetation, but the canopy cover (i.e. the quality of the cover), was negatively correlated to plumage damage in laying hens. This was most likely because of better range use in the group with good cover, which is known to reduce feather pecking and improve plumage condition (Bestman and Wagenaar, 2003; Nicol et al., 2003). Similarly, Zeltner and Hirt (2008) found that different structures in the range, including artificial and natural shelter, improved free-range use, but that the amount of range that was covered was not correlated to free-range use in laying hens. However, Bestman and Wagenaar (2003) and Dawkins et al. (2003) did find a relationship between coverage percentage and percentage of hens and broiler chickens outside. This indicates that both the amount of range covered and the quality of canopy cover could be of importance. Another factor that may be important is the direction and location in which the vegetation is planted. Birds are often observed to cluster around the pop holes, and it has been shown that shelter runways perpendicular to the poultry house were effective in improving the distribution of laying hens on the range (Pettersson et al., 2016b).

In addition to shelter on the range, shelter close or adjacent to the pop holes might be important. It has been suggested that transparent curtains between the house, winter garden (a covered range area) and free-range area, leading to small differences in light intensity between the areas, resulted in better free-range use in laying hens (Dekker et al., 2012). Also, in a study with 33 flocks of laying hens, a higher light intensity in the house, causing a smaller difference between inside and outside, was related to a higher percentage of hens using the range (Gilani et al., 2014). A more gradual transition from the house to the range, such as shelter adjacent to the pop holes, could make it possible for the birds to go outside but still be protected against e.g. bright light and birds of prey, while being able to scan the range and then venture farther from there.

Thesis focus 2: Short rotation coppice willows (SRCW)

When searching for an appropriate shelter type, taking into account the probable superiority of natural shelter in attracting chickens to the range, and the importance of canopy cover, a promising option is SRCW (Figure 1.1). The willow clones used in this production system are selected on fast growth and high biomass yield (Larsson, 1998). In practice, the trees are usually planted according to the 'Swedish system', which means they have a density of 15,000 trees / ha. SRCW is a dense vegetation, thus providing a lot of canopy cover, while at the same time not limiting space on the ground for the birds to walk, similar to jungle fowls' natural environment (Collias and Collias, 1967), especially when canopy closure occurs and undergrowth decreases due to the trees blocking the sunlight. SRCW is usually harvested every three years, and can reach a height of 8 m.

If chicken farmers would grow SRCW, they could use the wood chips for energy production on-farm, or sell them, thereby creating an extra income. Alternatively, they could also be used as litter in the chicken houses. A typical SRCW plantation has a total lifespan of 21 years thus can be harvested seven times. Average biomass yield depends inter alia on the clones that are used, but typically ranges between 10 and 16 tonnes dry matter (**DM**) / ha / year (Albertsson et al., 2016; Bergante et al., 2016; Stolarski et al., 2013).

In addition to providing shelter for the chickens and an extra source of income for the farmer, SRCW has other potential benefits. When the wood is used for energy production, this qualifies as renewable energy. Currently, the share of renewable energy production in Belgium is 7-8% (European Commission, 2017); this should be 13% in 2020 (European Commission, 2009) so there is still need for improvement. Biomass and renewable waste constitute the majority (75%) of renewable energy produced in Belgium (Eurostat, 2016), so SRCW could be included in this. In addition, if SRCW is produced on chicken ranges, the same land can be used to produce both chicken meat and biomass. This means the land is used more efficiently, and also improves farmers' economic resilience to an unfavourable market for one of the two products. Other favourable characteristics of SRCW may include an increased biodiversity (Baum et al., 2009; Sage, 1998), reduced NH_3 emissions (Adrizal et al., 2008; Patterson and Adrizal, 2005) and a reduced N leaching to groundwater due to uptake from deep soil layers (Bergström and Johansson, 1992; Dimitriou et al., 2012a; Goodlass et al., 2007). Together, these factors make it worthwhile investigating the implications of producing SRCW on a chicken range.

Figure 1.1 Short rotation coppice willows in the first year of a 3-year growing cycle.

1.3.2 Early-life experience

Some early-life experiences can have a persistent effect on animals' behaviour later in life. A vast number of studies have been performed on rodents. These show e.g. that environmental enrichment (such as running wheels, tunnels or toys) early in life induces a decrease of anxiety-like behaviour in different approach-avoid conflict tests (leading to more activity and/or exploratory behaviour; Fernández-Teruel et al., 2002; Holmes et al., 2005). This suggests that the enrichment influences brain development, mediating responses to novelty or conflict (Holmes et al., 2005). In addition, there are indications that fearfulness and anxiety are reduced if environmental enrichment is provided (Fernández-Teruel et al., 2002). It has e.g. been shown that enrichment can reduce hypothalamus-pituitary-adrenal (HPA) axis reactivity in rats that experienced prenatal stress (Morley-Fletcher et al., 2003). Another early-life-experience which may have substantial influence on animals' later life through influencing the HPA axis is maternal care. Rats that received more maternal care during the first 10 days of their life had lower levels of stress hormones and a better negative-feedback effect of glucocorticoids (Liu et al., 1997). Possibly, the negative effects of withholding maternal care depend on the genetic background of the animal, with more robust animals experiencing much less adverse consequences (Anisman et al., 1998). In addition to studies in rodents, the effects of early-life experience have also been tested in different farm animal species. With pigs, for example, social isolation between day 3 and 11 had long-term effects through changes in behavioural, neuroendocrine, and immune regulation (Kanitz et al., 2004). With laying hens, the presence of a mother hen during rearing increased chicks' foraging activity and led to decreased fearfulness and higher social motivation later in life (Perré et al., 2002; Riber et al., 2007; Roden and Wechsler, 1998). Housing and management conditions early in life could possibly also have long-term effects on the behaviour and welfare of broiler chickens.

For free-range or organic broiler chickens it is not mandatory to provide them with enrichment material during the rearing period (i.e. before they get outdoor access). They are often raised under quite barren conditions, which might not prepare them for the novelty of outdoor access later in life. Habituation to the range has to take place quite fast because the outdoor period is short (from 5 to 10 weeks of age). A study comparing broiler chickens given outdoor access at 4 or at 5 weeks of age found that both groups' free-range use increased with age, but that the increase was more pronounced in the groups given access at 4 weeks, showing that early experience could be of importance (Stadig et al., 2014). Early outdoor access may not be implemented by farmers due to a (perceived) decrease in production or increased risk of predation, but perhaps alternative rearing strategies other than early range access could also provide adequate preparation. Strategies aimed at reducing fearfulness appear to be promising, as this has shown to be negatively related to free-range use in laying hens (Campbell et al., 2016a; Hartcher et al., 2016; Mahboub et al., 2004), and can be influenced by early-life experiences.

There are different strategies to reduce fearfulness through altering early-life experiences, one of which may be environmental enrichment. Environmental enrichment is often defined as "a combination of inanimate and social stimulation" (Rosenzweig et al., 1978), although it has been argued that it should be beneficial for the animal and should thus be defined as "an improvement in the biological functioning of captive animals resulting from modifications to their environment" (Newberry, 1995). Social enrichment can be e.g. communal rearing (Branchi et al., 2006) or being housed with conspecifics (Bourgeois and Brent, 2005; Elliott and Grunberg, 2005). Non-social enrichment can be either physical, olfactory, auditory or visual. This thesis will focus on physical environmental enrichment. This can be e.g. providing species-specific feeding methods, adding biologically relevant structures such as perches, or providing objects for manipulation and exploration (Newberry, 1995). In this thesis, when referring to environmental enrichment, non-social enrichment is meant.

When broiler chickens were provided with different objects for pecking and perching between day 1 and 41, they had shorter tonic immobility (**TI**) durations on day 44 than broilers without those objects (Nicol, 1992). The TI test can be used to assess fearfulness; the chicken is placed on its back in a cradle, and the time to righting is recorded: longer durations indicate higher levels of fearfulness (Jones, 1986; Jones and Faure, 1981). Access to various objects was also associated with chicks being less immobile in an open field (**OF**) test and have shorter emergence latencies in a hole-in-the-wall box, which are also indicative of less fear (Jones, 1982). In laying hens, several studies showed that manipulable, brightly coloured objects, litter, short exposures to an outdoor area, and presence of a radio are able to reduce fearfulness (Jones and Waddington, 1992; de Haas et al., 2014; Grigor et al., 1995; Reed et al., 1993). However, not all studies found effects of enrichment on fear: mealworms (Pichova et al., 2016) or strings and perches (Bailie and O’Connell, 2015) were not successful in reducing broilers’ fear as measured with TI and novel object tests. Perhaps the provision of a small amount of mealworms once a day was not sufficient to elicit an effect. The possible effects of strings and perches may have been masked by the fact that straw bales were also present in all treatment groups, which may have been a preferred enrichment, facilitating pecking behaviour and acting as an elevated platform, hence serving the same purposes as the extra enrichments. More research is needed on which enrichments can be successful in decreasing fearfulness in broiler chickens.

Another possibly fear-alleviating strategy is rearing chicks with a broody hen or access to dark brooders. Dark brooders can also be considered a form of environmental enrichment, as they are a biologically relevant structure. In commercial practice, broiler chicks are reared without mother hens, which in laying hens led to higher levels of fearfulness at 4-8 weeks of age when compared to chicks that did receive maternal care (Campo et al., 2014; Roden and Wechsler, 1998; Rodenburg et al., 2009; Shimmura et al., 2010). However, keeping chickens of different ages (a mother hen with chicks) together can be a risk factor for animal and public health (Bouwknegt et al., 2004; Kleven, 2008; Medhanie et al., 2013). Additionally, broody hens may not only have a positive effect on chicks’ development: if the hens are fearful themselves, they may transmit this to the chicks (Houdelier et al., 2011). An alternative for the presence of hens could be dark brooders. These are raised panels, with heat elements underneath, and plastic fringes on the sides, creating a warm, dark space where resting chicks can seclude themselves.

So far, the use of dark brooders has only been tested in laying hens. They have been shown to reduce feather pecking (Gilani et al., 2012; Jensen et al., 2006; Riber and Guzman, 2016), probably because active and resting animals are separated during the period in which chicks learn which materials are appropriate for foraging and dust bathing; the inactive chicks are under the brooders and thus less likely to be pecked by the active animals (Riber, 2007). In addition, there are indications that the dark brooders cause chicks to be less fearful (Gilani et al., 2012; Riber and Guzman, 2016). However, the exact effects and mechanism remain unclear, and no studies with broiler chickens have been performed yet.

In addition to reducing fearfulness, environmental enrichment and dark brooders could also influence free-range use via other pathways. Enrichment may stimulate foraging behaviour in broilers because it is a more interesting and variable substrate than litter (Jones and Waddington, 1992; Pichova et al., 2016), it may increase general activity levels (Kells et al., 2001; Nicol, 1992), and it may positively affect learning performance (Krause et al., 2006) which could all contribute to a better free-range use later in life. Dark brooders may also teach chickens that exploration is rewarding, because by exploration they can discover the dark brooder as a place to rest, and the rest of the pen for feeding, foraging and interacting with conspecifics. In addition, the brooders could give the chickens more controllability over their environment, because they have more freedom to choose between different environments, which could positively affect their welfare through reduced stress levels (Wiepkema and Koolhaas, 1993).

1.3.3 Weather conditions

Weather conditions probably play an important role in free-range use. Although they cannot be influenced, it is important to know which conditions may be adverse to chickens. With that knowledge, range design could be adjusted to meet the chickens' needs and provide better protection from those adverse conditions. For example, if strong winds are experienced as adverse, this could be taken into account when designing the chicken house (direction of the pop holes) and the layout of the range (hedges could be planted to block the wind near the pop holes). Or, if high solar radiation causes the birds to range less, shelter which offers sufficient shade could be provided, such as vegetation or shade cloth.

Previous studies showed that, in temperate climates, broiler chickens ranged less in winter (average temperatures 3 – 15 °C) than in summer (average temperatures 14 – 30 °C), suggesting that low temperatures are adverse to broiler chickens (Dal Bosco et al., 2014; Dawkins et al., 2003; Jones et al., 2007), although other factors such as precipitation or depletion of vegetation may also be responsible for this. In summer months in the UK, increasing temperature, decreasing rainfall and days without sun positively affected the number of birds outside (Dawkins et al., 2003). A Spanish study found a positive effect of temperature only between 8 and 10 weeks of age (Rodriguez-Aurrekoetxea et al., 2014). Two studies showed that more broiler chickens were outside on sunny days or in a hot, sunny climate if trees or shade panels, respectively, were present on the range (Fanatico et al., 2016; Jones et al., 2007). This suggests that solar radiation may be adverse to them, but that shade may facilitate them to range anyway. This may be related to too high temperatures associated with high solar radiation. Alternatively, bright light may impede birds' ability to detect raptors. These studies give indications that low temperatures, rainfall and high solar radiation are adverse to broiler chickens, but the mitigating effect of shelter types has been tested insufficiently.

1.3.4 Other factors affecting free-range use

Group size It has not yet been studied in broiler chickens, but in laying hens it has been documented that larger groups generally use the range less (Bestman and Wagenaar, 2003; Gilani et al., 2014; Whay et al., 2007). Gebhardt et al. (2014) found no relationship between group size and the average number of hens outside, but in smaller flocks the proportion of hens frequently using the range was larger. The exact reasons for this remain unclear, but the smaller distance that needs to be crossed to reach the pop holes and go outside may play a role (Chielo et al., 2016). Mobile houses could therefore improve free-range use, because they often house relatively small groups of poultry, and perhaps also through more fresh air and light in the house, making the transition between the indoor and outdoor environment less abrupt. Another possible reason for better free-range use in smaller groups is an increased synchronization of behaviours in small flocks (Keeling et al., 2017), which could cause all birds in such flocks to go outside together during daytime. Alternatively, increased range use in smaller flocks may not be directly caused by the flock size, but by confounding factors such as the smaller total space allowance or a different microclimate in smaller houses (Gebhardt-Henrich et al., 2014).

Genetic strain Slow-growing broiler chickens, which are usually used in free-range production systems, generally go outside more and use more of the range than fast-growing strains (Castellini et al., 2002a; Dal Bosco et al., 2010; Nielsen et al., 2003). This is most likely related with poorer mobility due to impaired gait in fast-growing birds (Nielsen et al., 2003). However, genetic strain may also influence free-range use through differences in activity levels, fearfulness and exploration motivation. Castellini et al. (2002a) found longer TI durations in fast-growing Ross birds than in slower-growing Kabir or Robusta maculata chickens, but the reason for this remains unclear. The suggestion that genetic background and fearfulness are related is supported by studies with laying hens (Jones, 1977; Jones et al., 1995; Jones and Faure, 1981; Jones and Mills, 1983; Korte et al., 1997; Uitdehaag et al., 2008). Subsequently, these factors are also related to free-range use. Two genotypes of laying hens differed in fearfulness but also in free-range use, with higher levels of range use being associated with less fear (Mahboub et al., 2004). Additionally, it has been shown to be possible to influence ranging behaviour through genetic selection in laying hens (Icken et al., 2008). For broilers however, it is unclear how their genetic background might affect fearfulness and thereby free-range use.

Time of day Jungle fowl are known to have a diurnal rhythm in their activity; they are active and forage in the early morning and late afternoon / evening while they rest during the night and the middle of the day (Collias and Collias, 1967). A similar pattern can be found in domesticated broiler chickens with free-range access, with most birds being observed outside in these time periods (Christensen et al., 2003; Dawkins et al., 2003; Fanatico et al., 2016; Jones et al., 2007; Nielsen et al., 2003), although overall, broiler chickens are less active and rest more than jungle fowl (Cornetto and Estevez, 2001a).

Age of the birds

Generally, with age both the percentage of broiler chickens outside and their distance from the house increase (Christensen et al., 2003; Jones et al., 2007; Mirabito and Lubac, 2001). The increasing number of birds outside is probably due to habituation to the range or being less at risk of predation with increasing BW, while the increasing distance from the house could be due to habituation or to depletion of vegetation or other resources close to the house.

Indoor conditions In addition to the outdoor range providing favourable climatic conditions, range use could also be stimulated by adverse indoor climatic conditions, or agonistic interactions with conspecifics due to less space indoors. High dust and NH_3 concentrations for example, have been shown to have negative effects on bird health and laying hens choose fresh air over air with different concentrations of NH_3 (Homidan et al., 2003; Jones et al., 2005; Kristensen et al., 2000; Kristensen and Wathes, 2000). However, improving free-range use by deteriorating indoor conditions is not a desirable option from an animal-welfare point of view.

Pop hole size and design The effect of pop hole dimensions and design on free-range use has not yet been studied in broiler chickens. Studies with laying hens, however, give indications that free-range use increases with increasing pop-hole availability (cm/bird; Gilani et al., 2014; Sherwin et al., 2013). Elevated pop holes are sometimes used in laying hen houses, but these may be problematic for broiler chickens with impaired leg health.

Feed and water availability In commercial practice, feed and water are usually only available in the chicken house. Providing these resources on the range could attract more birds. This contains practical challenges such as other birds or animals such as rodents consuming the feed, which is a potential risk factor for transmission of diseases and an economic loss. However, systems that require the chickens to stand on a device in order to open the feeder do exist, and could solve this problem.

1.3.5 Conclusion

After reviewing existing literature on free-range use, it can be hypothesised that reasons responsible for limited free-range use are those displayed in Table 1.2. This table also contains possible remediating strategies aimed at improving free-range use.

Table 1.2 Potential reasons for low free-range use and possible remediating strategies

Reasons for low range use	Possible remediating strategies
Aversion to prevailing weather conditions	Adequate shelter on the range (SRCW?)
Fear of predators and/or new environment	Adequate shelter on the range (SRCW?)
	Gradual transition between indoor and outdoor
	Rearing method: reduce fearfulness
	Rearing method: early outdoor access
	Appropriate genetic strain
Low motivation to explore new environment	Rearing method: increase exploration motivation
	Provide suitable environment for exploration (SRCW?)
	Appropriate genetic strain
Social motivation / behavioural synchronization	Small group size
Physical inability (e.g. poor leg health)	Appropriate genetic strain (robust animals)
	Rearing method: stimulate activity
	Good litter quality
	No elevated pop holes
Motivation to stay in proximity of feed and water	Provide feed and water on the range
Time of day (low use at midday)	Provide outdoor access from early in the morning until the late afternoon or evening (depending on time of sunset)

1.4 How to monitor free-range use

In order to quantify the effects of free-range use and to test strategies to improve range use, it is important that the extent of free-range use is monitored well. In most studies monitoring is done through visual observations, i.e. the number of birds in predefined areas are counted at regular times, or focal birds are followed for fixed time periods. In general, such observations give a good indication of free-range use, but they also have several disadvantages. First, the presence of an observer can disturb the animals, that can either be curious and therefore moving towards, or fearful and moving away from the observer. Second, there is a trade-off between the time spent on observations and the amount of data that can be gathered. It is rarely feasible to perform observations during the entire free-range-access period, so specific time periods and / or days have to be selected to perform the observations. This, together with the diurnal rhythm of the chickens (outdoor use predominantly in the early morning and late afternoon / evening), hampers gathering results which are representative of the chickens' complete time budget. Third, the accuracy of visual observations may not be optimal. It will, for instance, be difficult to estimate the exact distance between a chicken and the house, or to their closest conspecific. Presence of vegetation such as shrubberies or trees on the range that impair visibility make this even more difficult. Last, it is difficult to monitor individual animals. A limited number of birds can be marked with colours or labels, but it is not feasible to monitor several thousand or even hundreds of individual birds. Until now, most factors influencing free-range use have been investigated at group level. This gives indications about factors playing a role in free-range use, but information on individual free-range use could elucidate more specific aspects playing a role in free-range use. It would, for example, enable researchers to more closely link free-range use to variables such as leg health, fearfulness, exploration or social motivation, range design, and meat quality. Therefore, research focussing on how to track individual chickens would be valuable.

In wildlife studies, different methods are used to track animals. One of these is the spool and line technique, which entails a thread being attached to an animal so that its tracks can be followed. Disadvantages of this technique are that it can interfere with normal movement patterns (Silvy, 2012), that the animal might get stuck in areas with dense vegetation, it has a limited range (depending on the length of the line) and it cannot be monitored how long an animal stayed at the places it visited. Other techniques such as bait-marking or rhodamine B ingestion leading to fluorescence of droppings cannot be easily used to track individual animals, and will therefore not be discussed further.

Using automated technologies to monitor individual free-range use is a potential solution for the difficulties posed by visual observations. An increasing number of studies are reporting the use of such systems. The most frequently used technology to date is Radio-Frequency Identification (**RFID**; Campbell et al., 2016a; Gebhardt-Henrich et al., 2014; Hartcher et al., 2016; Richards et al., 2011). An RFID system consists of transponders, which are usually attached to the chickens' legs, and horizontal antennas which register the transponders in their proximity. For studies on free-range use the antennas are situated in the pop holes, and the chickens have to pass them if they go outside. A disadvantage of this system is that the antennas can only read one transponder at a time, so if one chicken with a transponder is standing or sitting on the antenna, this can impede the registration of other transponders. This can be solved by using multiple smaller antennas instead of one large one, which can all separately register transponders, or by using anti-collision readers which make it possible to register more than one transponder within the reach of the antenna. Another difficulty is that the antenna cannot record the direction of the chicken, i.e. if it is going inside or outside. Therefore, if you assume that the first passage of the day is from inside to outside, the second one the other way around, and so on, and one passage is not registered, this means the data are no longer reliable. A solution for this is placing two consecutive antennas in each pop hole, so that the direction of a chicken can be deducted from the times both antennas are passed (Campbell et al., 2016b). In general, RFID enables gathering substantial amounts of data on individual birds, making it a valuable technology. Another novel system which uses light sensors to determine if hens are inside or outside is currently being developed; this system also has the potential to monitor the free-range use of a large number of individual birds (Buijs et al., 2017). The sensors are placed on the chickens' back, and the light intensity at this position is recorded. This intensity is then compared to light intensities measured by sensors at several fixed positions at the brightest areas of the house, in order to determine if a chicken is inside or outside.

A disadvantage of both the RFID and light sensor technology is that the exact position of the birds remains unknown. Knowing if birds are 1 or 100 m away from the house if they are outside would be valuable. Also, a system monitoring the exact location could serve to calculate distance to a birds' closest conspecific, which can be used for social network analysis, to monitor time spent in distinct outside areas, which can give indications of preferences for range design, or to monitor distance travelled, which can give indications about health status including lameness (Aydin et al., 2010; Weeks et al., 2000). One study applied global positioning system (**GPS**) technology to monitor the location of individual broiler chickens (Dal Bosco et al., 2010). However, the reported accuracy by the manufacturer of that system was 2.5 m. This may seem quite good, but when taking into account that most broiler chickens remain close to the house when outside, the need to consider a 'buffer zone' 2.5 m around the house could imply that for many chickens it is not certain that they are outside. Another study used a system with active tags, meaning they need an external power source (usually a battery). The tags send a signal to receivers placed at fixed positions, and the system calculates the distance between the tag and receiver based on time of arrival of the signal. Currently, such technology, combined with chickens, has only been applied indoors (Quwaider et al., 2010). Another system, that was designed to track positions of dairy cattle, made use of Ultra-Wideband (**UWB**) technology (Frondelius et al., 2014). This technology is currently also being used to monitor activity and distance to the closest conspecific in laying hens (de Haas et al., 2017). Both systems showed promising results, but again they were only tested indoors. Using it outside imposes several possible risk factors, such as the expected negative effects of water (Deak et al., 2010), meaning that water-containing objects such as chickens or leaves could have effect on signal transmission, which would have to be quantified.

1.4.1 Conclusions

Using visual observations to assess free-range use has several limitations, which could possibly be mitigated by using automated technologies. The main disadvantage of the currently used technologies is that they do not register the chickens' exact position. A system that could provide this information would be valuable because it can be used in many aspects of behaviour and welfare research.

Thesis focus 3: Automated positioning system (APS)

In this thesis, an UWB system will be developed in order to track the movement of individual broiler chickens housed free-range access. UWB systems are potentially capable of reaching accuracy of 10-50 cm (MacGougan et al., 2009). This will, however, depend on many factors, such as presence of other chickens and trees (both containing water), number and location of receivers, and perhaps also factors such as height, angle and orientation of the tag. It is therefore important to test the effects of these factors on both accuracy and signal reception.

1.5 Summary and research objectives

There is demand for meat from free-range broiler chickens. However, free-range broilers rarely use the range to its full potential, possibly due to a mismatch between the animal and the environment. Some likely reasons for low free-range use are adverse weather conditions, fear of predators or a new environment, and a low motivation to explore. A possible remediating strategy addressing these reasons is the provision of adequate shelter, possibly in the form of SRCW. This could provide benefits for the chickens as well as solutions for environmental challenges of outdoor access such as N leaching and C sequestration. A second strategy could be adjusted rearing methods, which could attenuate fearfulness, exploration motivation and leg health, and thereby also improve range use.

Improving free-range use probably has beneficial effects on the behaviour and welfare of broiler chickens, such as more freedom of choice, increased activity and better leg health. In addition, better range use could positively influence meat quality, although existing studies report conflicting results. With free-range chicken production there is a risk of high levels of N and P in the soil, leading to an increased possibility nutrients leaching to the groundwater. Strategies to prevent this, such as the use of mobile houses or vegetation capable of taking up these nutrients have to be studied further.

Range use can be monitored either with visual observations, which have several disadvantages regarding amount and accuracy of data gathered, or with technologies, which also have their limitations. Further development of such technologies would be really valuable for future studies on free-range use.

1.5.1 Research objectives and hypotheses

The overall aim of this thesis was to identify strategies to improve broiler chickens' free-range use, with a focus on SRCW as a shelter type capable of achieving this. The specific research objectives were:

1. To assess the effect of shelter type on free-range use (Chapter 2, 3, 4)
   Hypothesis: chickens prefer SRCW over artificial shelter, leading to more birds outside in this shelter type, and dispersing farther from the chicken house.
2. To assess the effect of weather conditions on free-range use (Chapter 2, 3)
   Hypothesis: rain, strong winds, cold and high solar radiation are aversive to chickens, resulting in a decrease in the number of birds outside.
3. To assess the effect of rearing strategy on fearfulness, behaviour and free-range use (Chapter 3, 4)
   Hypothesis: access to environmental enrichment or dark brooders early in life will reduce fearfulness, increase exploration motivation and lead to more free-range use at a later age.
4. To assess the relationships between free-range access & use and fearfulness & leg health (Chapter 2)
   Hypotheses: free-range access will result in better leg health and decreased fearfulness, and the more the birds use the free-range area, the larger these effects will be.
5. To assess the effects of free-range access and use on meat quality (Chapter 5)
   Hypotheses: free-range access will affect several chemical, physical and sensory characteristics of the meat, and the more the birds use the free-range area, the larger these effects will be.
6. To develop a system able to monitor free-range use of individual birds (Chapter 6)
   Hypothesis: birds' positions can be registered with a median error of 50 cm, but water-containing objects can hamper registration success, resulting in more missed registrations.
7. To assess interactions between presence of chickens, SRCW growth and soil nutrient balance (Chapter 7)
   Hypotheses: chickens may have a positive effect on SRCW growth through extra nutrient provision; soil will contain high levels of N and P close to the chicken houses; soil nutrient loads are smaller in SRCW than in grassland due to uptake from deeper layers by SRCW's rooting systems.

The conceptual framework (Figure 1.3) gives an overview of the focus of this thesis, in relation to other factors which are relevant for free-range use of slow-growing broiler chickens.

1.5.2 Thesis outline

Figure 1.2 depicts a timeline showing the different experiments and how these relate to the chapters in this thesis. In Chapter 2, the effects of free-range access on leg health and fearfulness, and the effects of shelter type and weather conditions on free-range use and leg health are discussed, by comparing groups of chickens kept exclusively indoors to groups kept with outdoor access to ranges with different shelter types. In Chapters 3 and 4, shelter types are again studied, but now chickens could choose between different types and hence show their preference. In addition, these chapters focus on the effects of different rearing strategies (environmental enrichment and dark brooders, respectively) and on behaviour of the chickens. Chapter 5 focuses on the effects of free-range use on production parameters and meat quality, by comparing the same groups of chickens as in Chapter 2. Chapter 6 discusses the possibilities and limitations of a newly developed APS which can monitor the location of chickens' with free-range access. Chapter 7 discusses the interactions between the presence of chickens, tree growth and soil nutrient balance. In Chapter 8, findings of the preceding research chapters are discussed and implications and limitations are formulated.

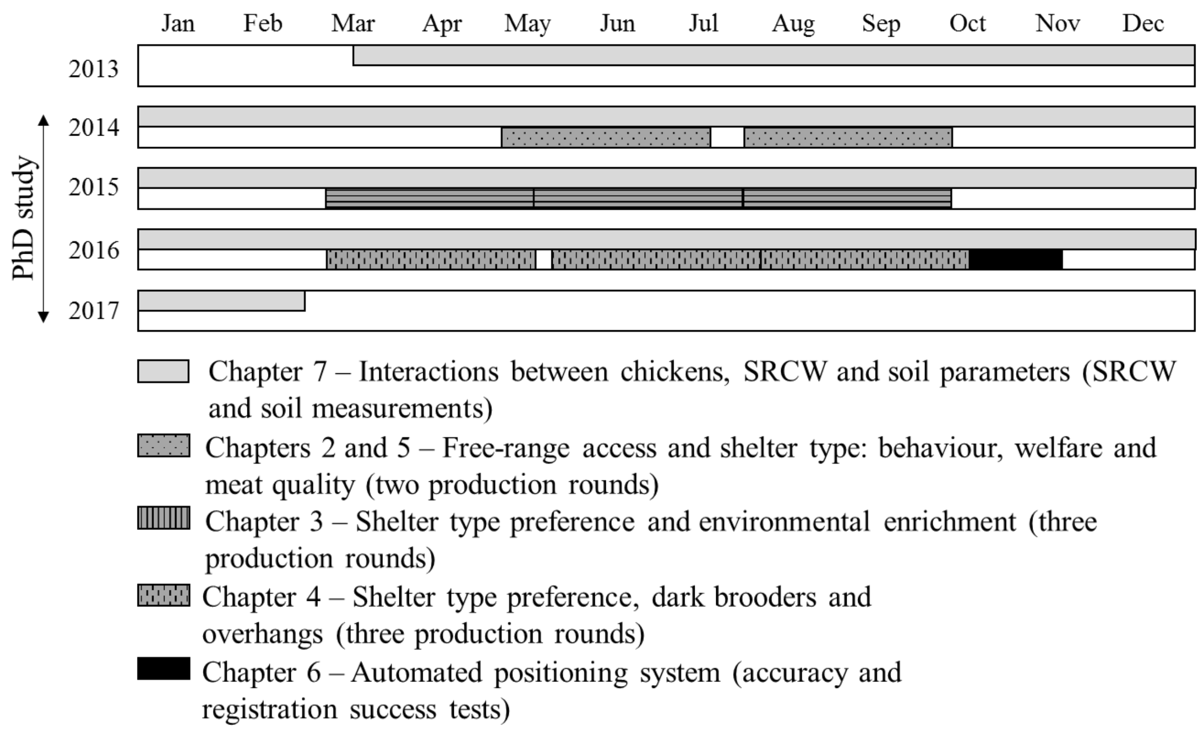


Figure 1.2 Overview of the different experiments and the chapters they are included in.

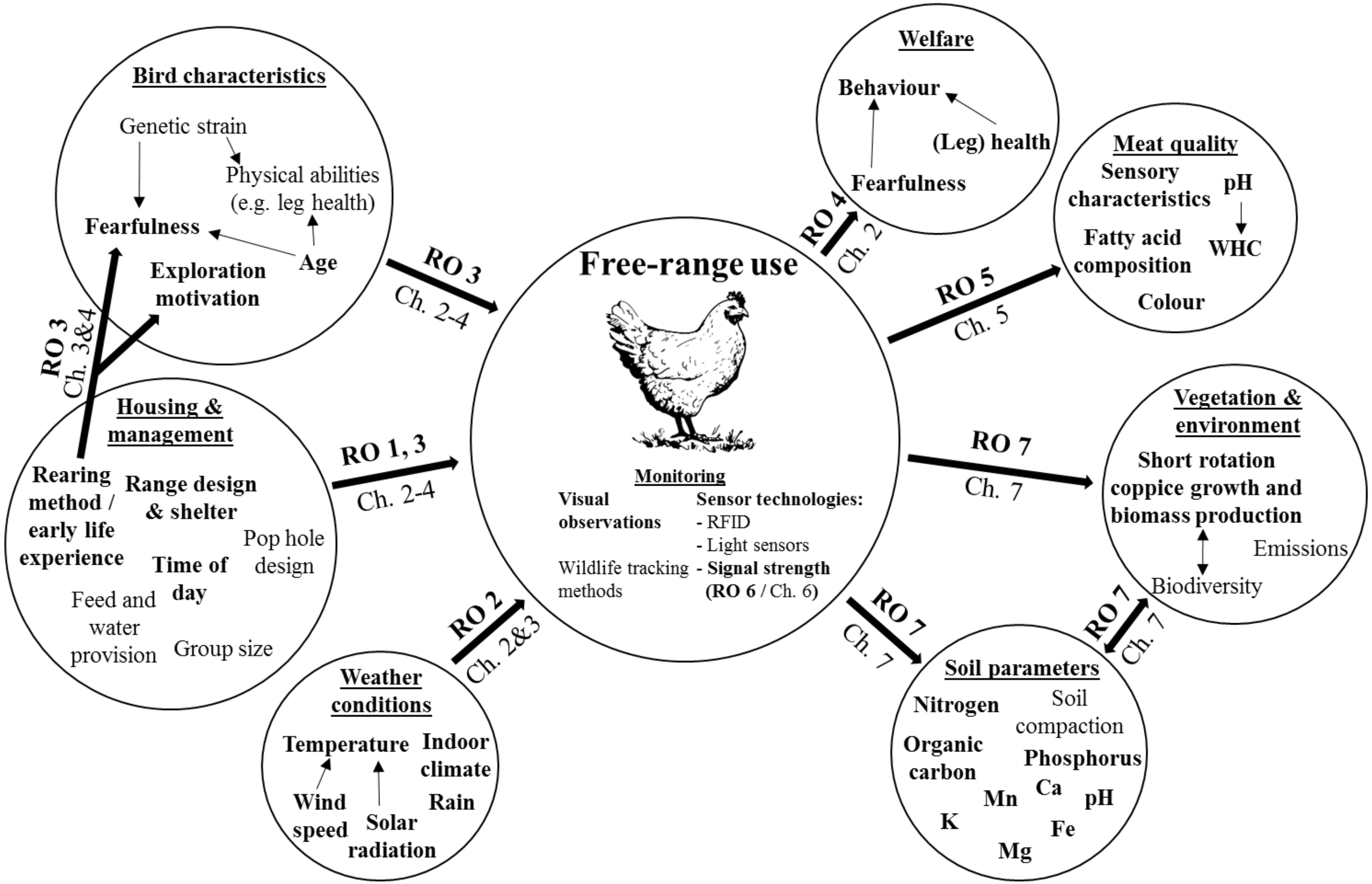
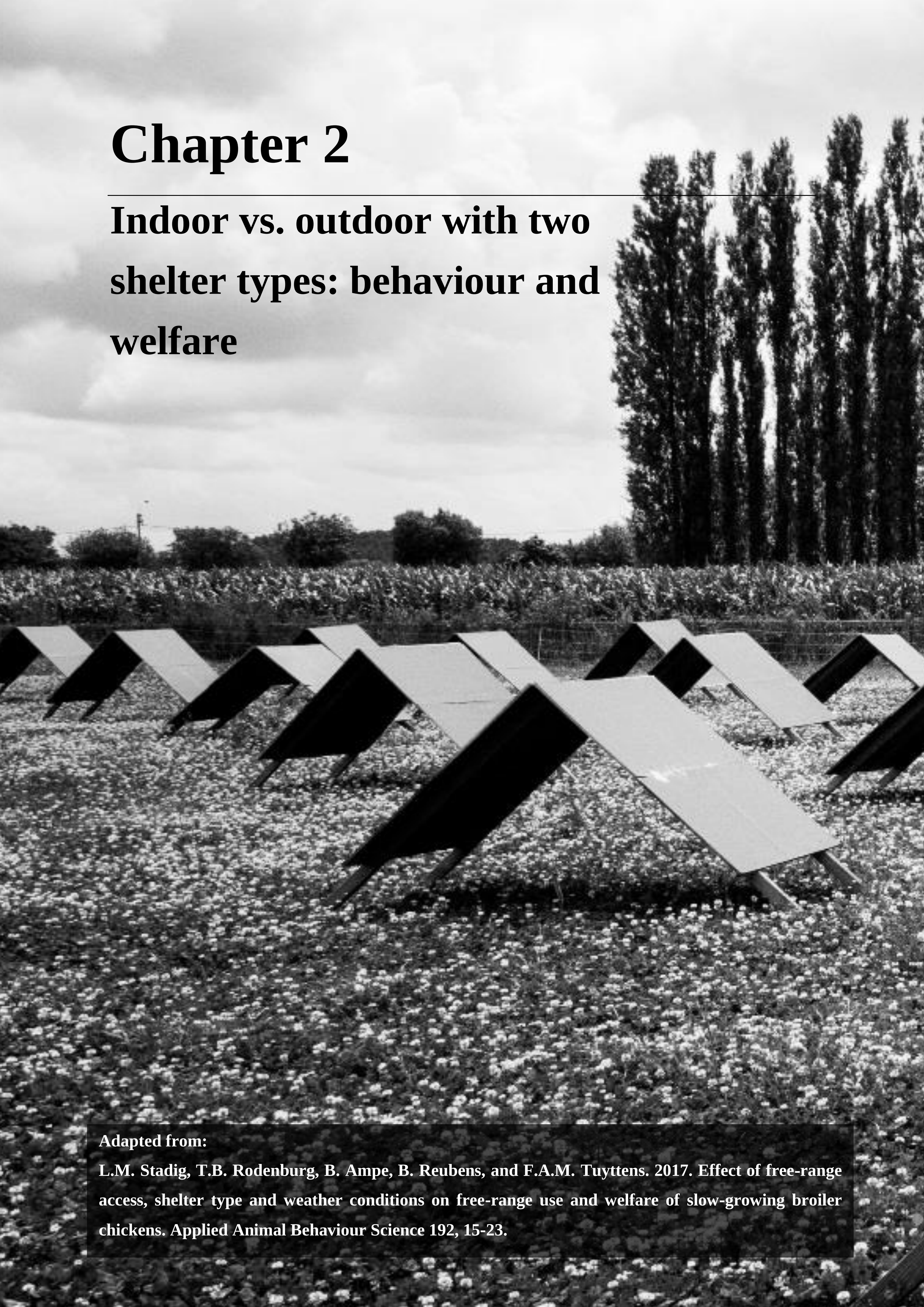


Figure 1.3 Conceptual framework illustrating the focus of this thesis with the research objectives (RO) and corresponding chapters (Ch.). Arrows indicate relationships between the variables. Parameters studied in this thesis are in bold. WHC = water-holding capacity; K = potassium; Mn = manganese; Mg = magnesium; Fe = iron, Ca = calcium.

Chapter 2

Indoor vs. outdoor with two shelter types: behaviour and welfare



Adapted from:

L.M. Stadig, T.B. Rodenburg, B. Ampe, B. Reubens, and F.A.M. Tuyttens. 2017. Effect of free-range access, shelter type and weather conditions on free-range use and welfare of slow-growing broiler chickens. Applied Animal Behaviour Science 192, 15-23.

Abstract

Free-range access for broiler chickens can benefit animal welfare because the birds have access to a more natural environment and more opportunities to perform natural behaviours than in indoor systems. Also, they have more space and more environmental enrichment, which could lead to better leg health and decreased fearfulness. In practice, however, use of the free-range area is often low. Lack of shelter likely plays an important role in this, as do weather conditions. In this study during 2 production rounds of slow-growing broiler chickens, 200 chickens were housed indoors (**IN**), 200 were provided with free-range access to grassland with artificial shelter (**AS**), and 200 were provided with free-range access to an area with SRCW from 4 until 10 weeks of age. Free-range use was monitored using photographs and live observations. Weather conditions and free-range use were monitored throughout the outdoor period. TI as fearfulness assessment was done at the beginning (round 2 only) and the end of both production rounds; leg health and tibia bone health were assessed at the end of the production rounds.

Mean percentage of birds using the free-range area was higher in SRCW than in AS groups (42.8% vs. 35.1%; $F_{1,7} = 1180.00$, $P < 0.001$). The mean percentage of animals located further than 5 m from the house was 10.6 ± 1.1% of the chickens that were outside in the SRCW groups vs. 4.1 ± 0.8% in the AS groups ($F_{1,7} = 24.03$, $P = 0.002$). The interactions of shelter type with rainfall ($F_{2,5578} = 70.59$, $P < 0.001$), increasing radiation ($F_{2,5578} = 300.93$, $P < 0.001$) and increasing wind speed ($F_{2,5578} = 14.77$, $P < 0.001$) showed that these factors were related with fewer chickens being outside; and that these effects were more pronounced in SRCW than in AS chickens. An increasing temperature was related with more free-range use ($F_{1,5578} = 32.24$, $P < 0.001$). A shorter TI duration in week 3 (at group level) was associated with more chickens further than 5 m from the house ($F_{1,250} = 13.79$, $P < 0.001$). The percentage of animals needing more than one induction to induce TI in week 10 was higher for chickens from SRCW (29.7%) than from IN groups (4.8%; $t_{102} = -2.61$, $P = 0.028$) but not AS (14.8%). Hock dermatitis occurred less in AS (7.6%) than in IN (40.1%; $t_{222} = 3.15$, $P = 0.005$) but not SRCW (13.7%). These findings indicate that presence of SRCW was most effective in encouraging chickens to use the free-range area, but that free-range access was only moderately related to better leg health and fearfulness (at group level).

Introduction

In many European countries, demand is growing for meat from broiler chickens that have had access to a free-range area (Ministerie EL&I, 2012; Verbeke, 2012). Although free-range access has possible disadvantages such as exposure to adverse weather conditions, risk of infection and exposure to predators, it is often considered to be beneficial for animal welfare (de Jonge and van Trijp, 2013; Vanhonacker et al., 2016). Reasons for this include access to a more natural environment and more opportunities to perform natural behaviours such as foraging and dust bathing (Knierim, 2006). Another positive effect of free-range access is additional space available to the chickens, which could lead to drier litter in the poultry house and in turn may lower the prevalence of foot pad and hock dermatitis (Ekstrand and Algers, 1997; Harms et al., 1977; Kyvsgaard et al., 2013). Further, the birds may get more exercise, particularly when the free-range area is attractive to them. This may lead to better leg bone development and fewer gait problems (Aguado et al., 2015; Leterrier et al., 2008).

Access to a free-range area can be seen as a form of environmental enrichment, which is associated with beneficial welfare effects, such as lower levels of fearfulness (in laying hens; Jones and Waddington, 1992). Laying hens that had free-range access and birds that frequented the free-range area (> 50% of all observations) tended to have shorter righting times in a TI test (indicative of less fear; Jones, 1986; Jones and Faure, 1981) as compared to those that were never observed outside (Grigor et al., 1995). Another study also found a negative relationship between TI duration and free-range use (Hartcher et al., 2016). These studies were performed with laying hens, however; it is not known whether free-range access also is related to fearfulness in broiler chickens. It is also not yet known whether less fearful chickens go outside more, or whether chickens that go outside more become less fearful over time.

Despite these possible advantages of free-range use the free-range area is not often used by chickens, particularly broiler chickens. Average percentages of broiler chickens outside at any given moment range from only 5 to 11% (Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). Broiler chickens may avoid the free-range area for the following reasons: lack of appropriate shelter (Dawkins et al., 2003), adverse weather conditions (Dal Bosco et al., 2014; Jones et al., 2007), young age (Jones et al., 2007; Mirabito and Lubac, 2001), unsuitable hybrid for free-range production (e.g. too fast-growing, which limits mobility; Nielsen et al., 2003), and personality characteristics such as fearfulness (Hartcher et al., 2016 (laying hens)).

Shelter is a key attribute of the range design (Dal Bosco et al., 2014; Dawkins et al., 2003) but little is known about the types of shelter preferred by broiler chickens in the free-range area. Domestic chickens descend from jungle fowl, thus it is likely that broilers will prefer an environment with similar dense vegetation. Such an environment allows birds to escape from aggressive conspecifics, and provides protection against adverse weather conditions and possibly increases the feeling of safety because birds of prey cannot attack through dense foliage, although 100% coverage could also be perceived as less safe because it could conceal predators (Newberry and Shackleton, 1997). It is also possible that an increased biodiversity in a forest-like environment compared to grassland is more attractive for chickens to display foraging behaviour. Which of these aspects of shelter are most important to the animals is not yet clear.

Shelter types can be divided into artificial and natural shelter. Artificial shelter can consist of e.g. vertical wooden panels, A-frames or camouflage nets. It has been found that indoors, vertical panels attract broiler chickens, leading to a more uniform use of space (Cornetto and Estevez, 2001b; Rodriguez-Aurrekoetxea et al., 2014) but this has not yet been replicated outside. Natural shelter can take the form of e.g. bushes, trees or tall grass. Each shelter type has its own advantages: natural shelter probably provides more biodiversity, while artificial shelter may protect better against rainfall. The type of shelter most preferred by the chickens is still unknown.

Several studies have shown that chickens prefer shelter from trees as compared to no shelter. Dawkins et al. (2003) found a relationship between the amount of tree cover in the range areas and the use of the free-range area by slow-growing broiler chickens. The same study revealed that when different types of vegetation were located equally far from the poultry house, broiler chickens preferred trees and bushes over grassland. Dal Bosco et al. (2014) found more range use in broiler chickens with access to olive trees than grassland. SRCW, i.e. coppices from fast-growing, high-yield tree species such as willow or poplar grown for biomass production, could act as suitable shelter for broilers. Willows are usually planted at high density, likely creating desirable shelter characteristics such as protection against adverse weather conditions and aerial predators. In addition to the function of sheltering chickens, SRCW can be harvested every 3 years. The wood chips can be sold, used for heat production, or used as litter material (Caslin et al., 2010).

Protection against adverse weather conditions is one important attribute of shelter for free-ranging poultry. Variables such as ambient temperature, wind speed and rainfall are known to be related to the use of the free-range area (Hegelund et al., 2005; Richards et al., 2012, 2011). As stated above, most studies have been performed with laying hens and it is not known whether these results can be extrapolated to broiler chickens, although Rodriguez-Aurrekoetxea et al. (2014) found an effect of temperature on free-range use, with a positive relationship between temperature and free-range use from week 8 to 10. The effects of certain weather conditions may also differ per climatic region, e.g. an increase in temperature may lead to more free-range use in temperate climates, whilst the opposite may be true for warmer climatic regions, depending on the range of temperatures that occur during the study.

The overall aim of this study was to gain insight into the effects of free-range access on welfare and to examine which factors influence free-range use. Specific goals were to investigate 1) how shelter type (SRCW (natural shelter) vs. AS) and weather conditions influenced free-range use, 2) whether (different levels of) free-range access had an effect on cleanliness and leg health in slow-growing broilers, 3) whether free-range access and shelter type influenced fearfulness, and 4) whether there was a relation between fearfulness and free-range use.

Materials and Methods

Animals and housing

In total, 1200 slow-growing (kept for 70 days as compared to 42 days for other hybrids such as Ross 308 (Tuyttens et al., 2014)) mixed-sex broiler chickens of the breed Sasso XL451 were used for the experiment. The Sasso hybrid was chosen because it is the most common hybrid in organic broiler production in Belgium, where nearly exclusively organic broilers get free-range access. During May-October 2014, two 10-week production rounds were performed with 600 chickens per round. Feed and water were available ad libitum. Feed was provided in three phases: starter feed from week 1 to 3, grower feed from week 4 to 7, and finisher feed from week 8 to 10. The feed was produced by ILVO (see Chapter 5 for the exact composition). All animal procedures were approved by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO, Merelbeke, Belgium).

All chickens were housed indoors from day 0 to 28 in round 1, or from day 0 to 21 in round 2. Each round included four groups of 150 animals (6 m^2 floor space per group; 25 birds per m^2).

Light schedule was 24L:0D for the first five days, and 16L:8D for the remaining days. Litter consisted of 10 cm of wood shavings.

For each round of 600 birds, 400 randomly selected birds (200 males, 200 females) were moved to four mobile chicken houses (2 groups per house, n = 25 males + 25 females per group) at day 28 and 21 in round 1 and 2, respectively. They were given free-range access from day 39 (round 1) or day 28 (round 2) until day 72. In round 1, the move date and start of free-range access were delayed due to construction problems with the flooring of the mobile houses. The remaining 200 chickens were housed indoors in four groups (n = 50 per group) until day 72 (IN). The light regime indoors (artificial light) was kept similar to the natural light regime. All housing systems were littered with 10 cm of wood shavings. All chickens, regardless of housing type, had the same amount of indoor space available (4 m^2 per group; 12.5 birds per m^2), and each pen contained one feeder and one bell drinker.

The four mobile houses were located on a 100 x 100 m plot (Figure 2.1). In each mobile house, one of the two groups of chickens was provided with free-range access to grassland with AS (21 wooden A-frames, l x w x h: 2.5 x 1.25 x 1.5 m; AS; Figure 2). The other group had access to Swedish SRCW clones (Tora (*Salix schwerinii*), Klara ((*S. burjatica* x *S. viminalis*) x *S. burjatica*) and Tordis (*S. schwerinii* x *S. viminalis*)) planted in spring 2013 at high density (15,000 trees/ha), in accordance with common practice (75 cm between single rows, 150 cm between double rows, 60 cm between trees in each row; SRCW; Figure 2.2). Each group had one 2 x 0.5 m pop hole opened in the morning between 0700 h and 0900 h and closed at sunset. The houses were repositioned between the production rounds to assure use of the entire area and to minimise point pollution.

Data collection

Free-range use The number of chickens inside the mobile houses was recorded with cameras (Bushnell Trophy Cam, Bushnell, Kansas City, Missouri). One photograph per hour (during the times the pop holes were open) was taken and used to calculate the percentage of animals outdoors at that moment. These data were used to analyse which factors influenced the number of birds going outside per treatment. Additional live observations were performed by one of the authors on 10 days in round 1 (day 43, 45, 50, 51, 52, 56, 57, 59, 63, 65), and 13 days in round 2 (day 29, 31, 36, 37, 38, 42, 43, 44, 49, 50, 51, 57, 58). The number of chickens using the free-range area was recorded per group (always at 0900 h, 1300 h and 1700 h) with a distinction

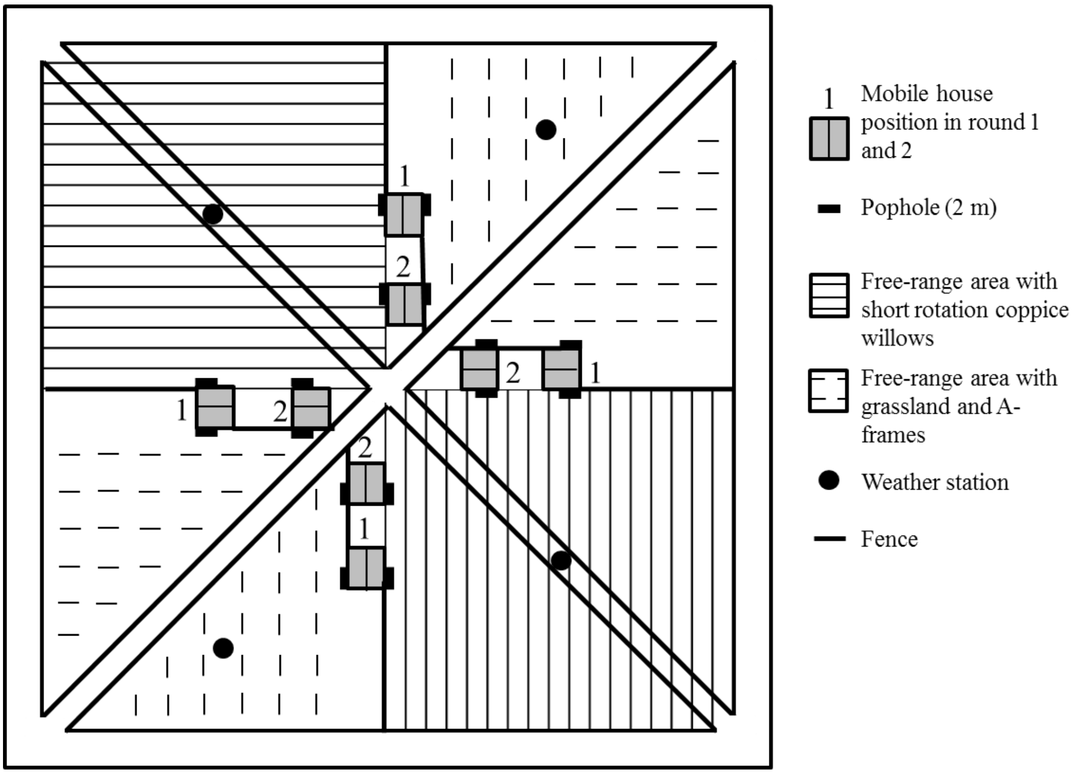


Figure 2.1 Top view of the experimental site (100 x 100 m). The numbers next to the mobile houses indicate the positions of the houses in round 1 and 2, respectively.

Figure 2.2 Left: free-range area with wooden A-frames. Right: free-range area with short rotation coppice willows.

between birds located within 5 m from the house and those further away. These data were used to analyse which factors influenced birds' distribution over the free-range area in addition to merely the percentage of birds that was outside, as derived from the indoor photographs. These measurements were not done blind, as both on the photographs as during live observations it was clear in which treatment group the chickens were.

Fearfulness The TI test was used to assess fearfulness. This test was performed in week 10 of both rounds. In round 1 60 birds (30 males, 30 females), in round 2, 120 birds (60 males, 60 females) were tested (TI trial 2). All birds were tested just in front of their home pen, and the assessor was not blinded from the treatments. In round 2, an additional test was done with 60 chickens in week 3 (TI trial 1). This trial was added because it was hypothesised that less fearful chickens may be more prone to use the free-range area, while the second trial was mainly to examine how free-range access influenced fearfulness. The test was carried out by the same researcher for all chickens in all rounds. The test itself consists of placing a chicken on its back in a U-shaped cradle and restraining it for 15 s. The chicken is then released and the time until righting (latency) was recorded. If a chicken righted itself within 10 s after restraint, another 15-s induction was performed with a maximum of three inductions. If all inductions were less than 10 seconds, a latency of 0 s was assigned. Chickens that did not right themselves within 5 min were assigned a latency of 300 s. After the TI test, each bird was weighed using a bucket and a hanging scale.

Cleanliness and leg health In week 10 (end of the production round) one assessor scored cleanliness and leg health parameters on 120 animals per round (n = 40 per treatment, 10 per group). The assessment took place on the experimental field, and the assessor was not blinded from the treatments. From each pen, 5 male and 5 female birds were selected based on their weight. This had to be within 0.2 kg from the average weight of all birds of their sex (determined during the TI tests). The birds were selected for weight because weight can influence parameters such as hock dermatitis and gait (Baéza et al., 2012; Broom and Reefmann, 2005) as well as meat quality parameters (Berri et al., 2001) which were determined on 48 of these birds (Chapter 5 of this thesis). The scored parameters were hock dermatitis, FPD, gait and cleanliness. The first three scores were performed as described in the Welfare Quality® protocol for broilers (Welfare Quality®, 2009). In short, hock dermatitis and FPD were scored on a 5-point scale, ranging from no to severe lesions, and gait on a 6-point scale, ranging from normal gait to incapable of walking. The scoring system for cleanliness was also based on the Welfare Quality® protocol, but word descriptors were added to all scores (0 = clean, 1 = slightly dirty breast but no clumps, 2 = dirty breast with clumps, 3 = dirty, wet breast).

Bone health After slaughter, both legs of 126 birds (the same birds as for scoring of cleanliness and leg health, plus six extra (needed for later analysis on meat quality; Chapter 5 of this thesis); 42 per treatment, 21 males and 21 females) per round were stored at -20 °C for 7 and 4 months for rounds 1 and 2, respectively. They were then thawed at 4 °C for 24 h before dissecting the tibia from each leg. Each bird's left tibia was placed in a 95 °C water bath for 15 min, after which any excess flesh could be removed using a toothbrush, then the tibia was dried in a desiccator at 25 °C for 24 h. A shear force test was then performed using a Basic Force Gauge 2500N (VersaTest Mecmesin, Slinfold, UK) according to ASABE Standards (ASABE, 2007). Crosshead speed of the apparatus was set at 12 mm / min. After the shear test, the shaft width (2 positions) and the wall thickness (4 positions) were measured on the proximal half of the tibia using digital callipers (Mitutoyo, Kruibeke, Belgium). These were used to calculate shear stress (force per unit area) according to Combs et al. (1991).

The right tibia was placed in a 65 °C water bath with 50 g sodium perborate ($NaBO_3$) / L for 19 h, after which it was cleaned with a toothbrush and dried in a desiccator for 21 h at 25 °C. The weight of the tibia was recorded and length was measured using digital callipers. The degree of torsion was recorded by measuring the angle of the distal epiphysis to the horizontal plane when the proximal epiphysis was fixed horizontally (Butterworth, 2001). Curvature was recorded on a scale of 0 to 3, ranging from straight to severely curved (Butterworth, 2001). Tibias were then sawed in half, 1 mm distal from the *foramen nutricium*. Marrow from the proximal part was removed using a small brush and shaft outer (2 positions) and inner width (2 positions) plus wall thickness (4 positions) were measured. The proximal epiphysis was sliced vertically to score tibial dyschondroplasia on a scale from 0 to 3 (Merck Veterinary Manual, 2012). The tibias were then stored at -20 °C for 2 months. Forty-eight (n = 24 per round) of them were thawed at 4 °C and any lipids were extracted using the ISO 6492:1999 method with petroleum ether. They were dried at 103 °C for 4 h then weighed. To determine the ash content, the tibias were placed in a muffle furnace at 825 °C overnight (ca. 16 h) before being weighed again. Both assessors were blinded to treatment for all bone health measurements.

Climatic conditions Weather conditions were recorded every 15 min using four weather stations, two of each were placed on the grassland, and two in the SRCW (Figure 1). The weather stations were equipped with a thermometer and humidity meter (CS215, Campbell Scientific, Logan, UT, USA), a precipitation meter (ARG100 Tipping Bucket, Campbell Scientific), a pyranometer (CS300, Apogee Instruments, Logan, UT, USA), a wind speed meter

(03002 Wind Sentry Set, R.M. Young, Traverse City, MI, USA), and a data logger (CR200, Campbell Scientific). The recorded parameters were temperature (°C), humidity (%), rainfall (mm in the preceding 15 min), radiation (kW/m^2), and wind speed (m/s). Rainfall data were dichotomised (0 = dry (0 mm rain/15 min), 1 = rain (>0 mm rain/15 min)). Climatic indices (Heat Index (**HI**), the Wind Chill (**WC**), and the Dew Point Temperature (**DPT**)) were calculated using the software in the weather stations (Short Cut, Campbell Scientific, Logan, UT, USA). HI is the perceived temperature as influenced by the relative humidity. That equation is only useful when air temperature is above 27 °C and relative humidity is above 40%, thus the program will set the HI temperature equal to the current air temperature if these conditions are not met. WC is the perceived temperature as influenced by wind speed. The DPT is the temperature at which water vapour starts to condense out of the air. The exact equations of all indices can be found on the Campbell Scientific website (Campbell Scientific, 2001). Indoor climate was also measured; data loggers (Testo, 177-H1, Sparta, NJ, USA) were placed in each of the mobile houses to record temperature (°C) and humidity (%).

Data analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). For all models, non-aggregated data were used because the analysis of aggregated data would be confusing since the level of aggregation would depend on the factors included in the model. This also means that inclusion of two factors in the same model would be impossible or would need an aggregation on the combination of e.g. day and temperature level which would be difficult to interpret. The percentage of birds outside was analysed using a linear mixed model (proc glimmix) with shelter type and additional variables (described below) as fixed effects. The percentage of outside birds further than 5 m from the house was analysed using a mixed logistic regression model (proc glimmix) with shelter type and additional variables (described below) as fixed effects. In all cases AS, SRCW and IN were included in the analyses as shelter type. However, when the fixed factor ‘average percentage outdoors’ was included in a model (for fearfulness, cleanliness and leg health, and bone health), the results for IN were not relevant and therefore excluded. Appropriate measures were taken to correct for the different types of dependence in the data. House (n = 4) within production round (n = 2) was included as random effect in all models. To account for the repeated measures over time, a first-order autoregressive covariance-structure was used in the mixed models. Binary variables (for variables fearfulness, cleanliness and leg health, and bone health) were analysed using a similar mixed logistic regression model. Continuous variables were considered sufficiently normally distributed based on the graphical evaluation (histogram and QQ plot) of the residuals. Statistical significance was evaluated at $\alpha < 0.05$. Factors and interactions with a P value > 0.1 were removed from the final models, starting with those with the highest P value in the type III F-test. Data are presented as (back-transformed) least squares means (**LS means**; which is a proportion, multiplied by 100 to get a percentage) and their standard error or 95% confidence limits unless stated otherwise. In case of post-hoc pairwise comparisons, the Tukey-Kramer adjustment for multiple comparisons (t-test) was used at a total significance level of 0.05.

Free-range use It was tested which factors influenced free-range use. The percentage of animals outside ((number outside (derived from the photographs) / total number of animals) x 100), and percentage of the outside chickens positioned more than 5 m from the house ((number of animals further than 5m (derived from the live observations) / number of animals outside) x 100), were analysed. The initial fixed factors were shelter type, climatic parameters (temperature, humidity, rainfall, solar radiation and wind speed) and their indices (HI, WC and DPT), age, and interactions between age, climatic parameters and shelter type. House within round was added as random factor to correct for repeated measures within the pen (for percentage of animals outdoors: 1 observation each hour during the times the pop holes were opened; for outside birds further than 5 m from the house: three times daily on 10 or 13 days in round 1 and 2, respectively). Outdoor climatic parameters from the weather station on each particular field were used, the recording made closest to the moment of observation was used. Some climatic parameters were strongly correlated (R > 0.7) with each other, allowing for only one of them (i.e. the most significant) to be used in the final model. A separate model was built which also included TI duration and number of inductions for TI trial 1 as explanatory factor for free-range use. This trial was performed in the second production round only, thus including it in the previous model would mean losing half of the data. Both final models for the percentage of birds outside (with and without TI as initial fixed factor) included the following fixed factors: shelter type, rainfall x shelter type, solar radiation x shelter type, wind speed x shelter type, temperature and age (TI was not included as its effect was not significant). The final model for the percentage of outside chickens positioned more than 5 m from the house included the following fixed factors: shelter type and radiation. When including TI, the final model for this variable included these fixed factors: shelter type, radiation and TI (trial 1) duration.

Fearfulness It was tested which factors influenced birds' fearfulness as measured in week 10 (TI trial 2; n = 60 birds in round 1 and 120 in round 2). TI data did not follow the normal distribution, therefore TI durations were log-transformed, and number of inductions were dichotomised (0 = one induction, 1 = more than one induction). Fixed factors of the linear mixed model were shelter type and sex. House within round was added as random factor to correct for repeated measures within the pen (5 per pen in round 1, 10 in round 2). Within shelter type, a mixed model was used to assess if the average percentage of birds outdoors (on group level) was related with the TI duration or number of inductions in trial 2, using the average outdoor percentage and sex as fixed factors.

Cleanliness and leg health It was tested which factors influenced birds' cleanliness and leg health (gait, FPD, hock dermatitis) as measured in week 10 (n = 120 birds per round). Because cleanliness and leg health scores did not follow a normal distribution, they were dichotomised with scores > 1 being converted to 1 and analysed using a mixed logistic regression model with shelter type and weight of the bird as fixed factors. House within round was added as random factor to correct for repeated measures within the pen (10 per pen per round). Within shelter type it was assessed if the average percentage of birds outdoors (on group level) was related with leg health and cleanliness parameters using the average outdoor percentage and bird weight as fixed factors.

Bone health It was tested which factors influenced birds' tibia health (shear stress, weight, length, torsion, curvature, outer and inner width, wall thickness, dyschondroplasia, ash content; n = 126 birds per round, except for ash percentage (n = 24 per round)). One outlier for bone wall thickness and two outliers for shear stress, based on the Cook's Distance and the difference in fits (**DFFITS**), were removed from the data set. Bone health data were analysed using a mixed model with shelter type and sex as fixed factors. House within round was added as random factor to correct for repeated measures within the pen (10 or 11 per pen per round, 4 per pen per round for the ash percentage). Because the curvature of tibias was not normally distributed, the curvature of tibias was dichotomised with scores > 1 being converted to 1, and analysed using a mixed logistic regression model with shelter type and sex as fixed factors. Within shelter type it was assessed if the average percentage of birds outdoors (on group level) was related with bone health parameters using the average outdoor percentage as fixed factor.

Results

Free-range use

Free-range use was related to shelter type, age and climatic conditions. Mean percentage of animals outside was higher for SRCW than for AS chickens ($F_{1,7}$ = 233.7, P < 0.001; Figure 2.3). An overview of the means and ranges of the recorded climatic parameters and indices is given in Table 2.1. Rain, increased radiation and increased wind speed were all related to fewer chickens observed outside, and these effects were more pronounced in SRCW than in AS chickens (rain x shelter type: $F_{2,5578}$ = 70.59, P < 0.001; radiation x shelter type: $F_{2,5578}$ = 300.93, P < 0.001; wind speed x shelter type: $F_{2,5578}$ = 14.77, P < 0.001; Figure 2.4). Nevertheless, for the most prevalent weather conditions in this study, the percentage of chickens outside was highest in the SRCW groups. An increase in temperature was linked to increased outdoor use ($F_{1,5578}$ = 32.24, P < 0.001; Figure 2.4). The percentage of chickens outside increased by 0.3% per day ($F_{1,5578}$ = 79.29, P < 0.001). The analysis including TI trial 1 showed no relations at group level between mean TI duration or number of inductions and free-range use ($F_{1,3037}$ = 1.67; P = 0.196 and $F_{1,3037}$ = 1.43; P = 0.232, respectively). The means and ranges of the TI test data are presented in Table 2.2.

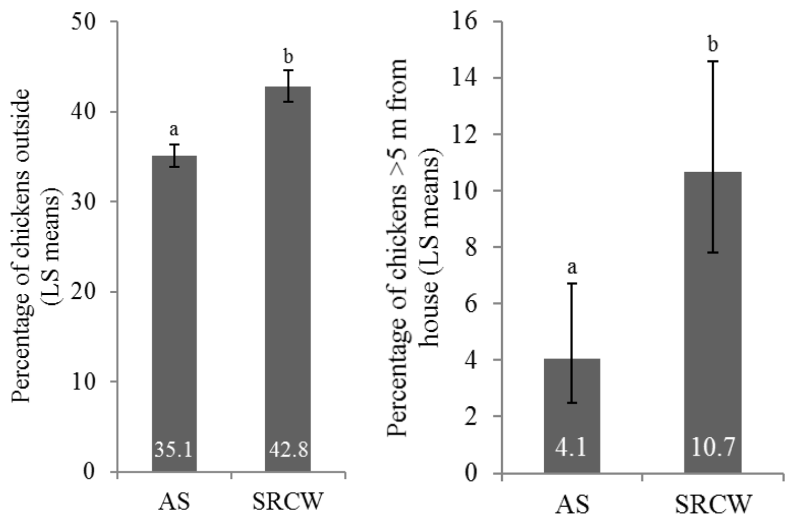


Figure 2.3 Percentage of chickens (LS means) outside (left) and further than 5 m from the house (right) in artificial shelter (AS) and short rotation coppice willow (SRCW) groups. Columns without a common letter differ significantly (P<0.05). CI = confidence interval.

Table 2.1 Mean and range of climatic parameters in the free-range areas with artificial shelter (AS) and short rotation coppice (SRC) and in the mobile houses during the weeks of free-range access.

Climatic parameter	Mean		Minimum		Maximum	
	AS	SRC	AS	SRC	AS	SRC
Outdoor temperature (°C)	15.9	16.2	5.0	5.2	28.3	29.2
Outdoor RH[1] (%)	89.1	92.3	35.0	35.2	100.0	100.0
Rainfall[2] (mm/15 min)	0.03	0.02	0.00	0.00	15.20	13.40
Wind speed (m/s)	0.6	0.2	0.0	0.0	4.2	3.8
Radiation (kW/m^2)	0.13	0.08	0.00	0.00	1.01	1.00
Heat Index	15.9	16.2	4.38	5.15	30.2	30.6
Wind Chill	15.9	16.2	4.38	5.15	28.3	29.2
Dewpoint Temperature	13.8	14.8	4.38	5.15	23.1	22.6
Indoor temperature (°C)	20.6		12.0		30.3	
Indoor RH (%)	74.3		31.3		99.9	

[1] RH = relative humidity. [2] Rainfall data were dichotomised prior to analysis.

Table 2.2 Mean and range of tonic immobility (TI) duration and number of inductions on group level

Parameter	Mean	Minimum	Maximum
TI trial 1 – mean duration (s)	130	72	198
TI trial 1 – mean number of inductions	1.3	1	1.6
TI trial 2 – mean duration (s)	153	68	220
TI trial 2 – mean number of inductions	1.2	1	1.6

The percentage of outdoor chickens further than 5 m from the house was influenced by shelter type and radiation. In SRCW groups, on average more of the chickens that were outside were located farther than 5 m from the house, than in AS ($F_{1,7}$ = 24.0, P = 0.002; Figure 2.3). Radiation was the only climatic parameter that influenced the number of chickens further than 5 m from the house, with more radiation being related to fewer chickens in this area ($F_{1,401}$ = 18.9, P < 0.001). For example, in an SRCW group, the mean percentage of outdoor chickens further than 5 m from the house would be 15.3% (95% CI: 11.3 – 20.3%) with a radiation of 0.1 kW/m^2, and only 1.3% (95% CI: 0.3 – 6.6%) with a radiation of 1.0 kW/m^2.

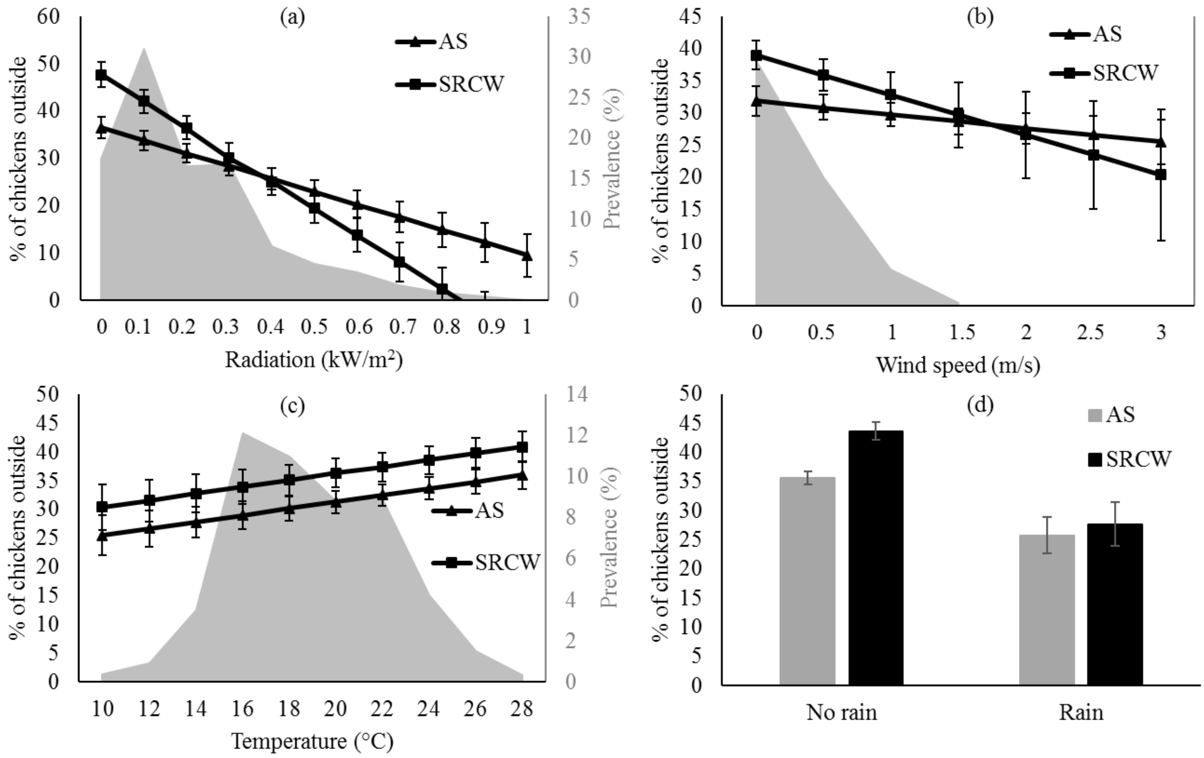


Figure 2.4 Relationships (based on LS means) between the percentage of chickens outside and (a) radiation, (b) wind speed, (c) temperature and (d) rainfall for birds in short rotation coppice willows (SRCW) and on grassland with artificial shelter (AS). The grey areas (right y-axis) in a-c represent the frequency distribution of the radiation, wind speed and temperature. The prevalence of rainfall was 1.7%. The error bars represent the 95% CI.

The analysis including TI trial 1 gave similar results for shelter type and radiation as the model without TI, and indicated that a longer mean TI duration in trial 1 (on group level) was associated with fewer outside chickens further than 5 m from the house ($F_{1,250}$ = 13.79, P < 0.001). For example, in the SRCW group with a mean TI trial 1 duration of 75 s, 29.3% (95% CI: 14.6 – 50.2) of the outside chickens were more than 5 m from the house, for a mean TI duration of 150 s this was 12.6% (95% CI: 7.3 – 20.8) on average.

Fearfulness

The duration of TI in trial 2 did not differ between treatment groups ($F_{2,102}$ = 2.2; P = 0.12). The number of inductions needed did differ between treatment groups ($F_{2,102}$ = 3.83; P = 0.025). The percentage of animals needing more than one induction to induce TI in trial 2 was higher in SRCW groups than in IN groups (29.7% (95% CI: 14.5 – 51.2%) vs. 4.8% (95% CI: 1.1 –

19.6%); t_{102} = -2.61; P = 0.028), but AS (14.8% (95% CI: 5.7 – 33.4%)) did not differ from IN (t_{102} = -1.43; P = 0.331) or SRCW (t_{102} = -1.58; P = 0.118). Within treatment groups, there was no effect of average free-range use on TI duration or number of inductions.

Cleanliness and leg health

There were differences in the occurrence of imperfect gait, hock dermatitis, and poor cleanliness (all with a score ≥1) between the IN, AS and SRCW groups (Table 2.3). Chickens from IN groups tended to have an imperfect gait more often than those from AS (t_{222} = 2.2; P = 0.077). Within the SRCW group, a higher mean percentage of free-range use tended to be related to fewer gait problems: a 1% increase in free-range use tended to equal a 4.1% decrease in imperfect gait (F_{71} = 2.9; P = 0.096). More hock dermatitis was observed in IN than in AS groups (t_{222} = 3.2; P = 0.005) and a tendency for more hock dermatitis was found for SRCW groups (t_{222} = 2.3; P = 0.061). Birds from AS groups also tended to be cleaner than those from IN groups (t_{222} = 2.3; P = 0.064). Higher weight was related with increased imperfect gait (F_{222} = 11.6; P < 0.001), increased hock dermatitis (F_{222} = 34.1; P < 0.001) and poorer cleanliness (F_{222} = 18.0; P < 0.001).

Bone health

Of the bone health parameters, only tibia length was related with treatment ($F_{2,227}$ = 3.47; P = 0.033): animals from IN groups had longer tibias than those from SRCW groups (124.57 mm (95% CI: 122.97 – 126.17) vs. 122.76 mm (95% CI: 121.16 – 124.36); t_{227} = 2.0; P = 0.026) but not than those from the AS groups (123.19 mm; 95% CI: 121.59 – 124.79). For chickens from AS groups, there was a relationship between free-range use and torsion and ash percentage of the tibia. A 1% increase in mean free-range use at group level tended to be related with a decrease of 0.16 degrees of torsion ($F_{1,73}$ = 3.24; P = 0.076) and with a 0.8% decrease in ash percentage ($F_{1,8}$ = 4.68; P = 0.062). These relationships were not found for the SRCW groups ($F_{1,75}$ = 0.83; P = 0.364 and $F_{1,8}$ = 0.30; P = 0.598, respectively).

Table 2.3 Occurrence (LS means and (95% confidence interval)) of leg health-related welfare problems and poor cleanliness in the indoor (IN), artificial shelter (AS) and short rotation coppice willow (SRCW) groups.

	IN	AS	SRCW	DF (nominator, denominator)	F value	P value
Imperfect gait	43.4%^{A} (24.1 – 64.9)	25.6%^{B} (12.1 – 46.1)	26.8%^{AB} (12.9 – 47.5)	2, 222	2.97	0.053
Foot pad dermatitis	81.2% (65.9 – 90.6)	71.8% (54.5 – 84.4)	67.9% (50.1 – 81.6)	2, 222	0.84	0.434
Hock dermatitis	40.1%$^{a;A}$ (22.6 – 62.1)	7.6%$^{b;B}$ (2.9 – 18.8)	13.7%$^{ab;B}$ (5.8 – 29.2)	2, 222	4.95	0.008
Poor cleanliness	88.0%^{A} (69.5 – 96.0)	71.9%^{B} (46.1 – 88.4)	84.3%^{AB} (63.1 – 94.4)	2, 222	3.16	0.045

Values within rows without common superscript differ significantly (P<0.05) or tend to differ (capital letters; P<0.1). DF = degrees of freedom.

As compared to females, males had both heavier (19.3 g (95% CI: 18.2 – 20.3) vs. 12.2 g (95% CI: 11.2 – 13.2); t_{225} = 33.5; P < 0.001) and longer (128.9 mm (95% CI: 127.4 – 130.4) vs. 118.1 mm (95% CI: 116.7 – 119.6); t_{227} = 23.3; P < 0.001) tibias, which had greater inner (7.59 mm (95% CI: 7.48 – 7.70) vs. 6.64 mm (95% CI: 6.53 – 6.75); t_{229} = 12.3; P < 0.001) and outer (10.79 mm (95% CI: 10.50 – 11.08) vs. 9.15 mm (95% CI: 8.85 – 9.44); t_{199} = 19.2; P < 0.001) diameters, thicker walls (1.58 mm (95% CI: 1.44 – 1.72) vs. 1.22 mm (95% CI: 1.08 – 1.36); t_{228} = 11.8; P < 0.001), a higher breaking strength (1156.7 N (95% CI: 1117.5 – 1195.9) vs. 927.5 N (95% CI: 888.5 – 966.4); t_{222} = 10.4; P < 0.001) and lower ash percentages (47.6% (95% CI: 34.1 – 61.1) vs. 51.7% (95% CI: 38.2 – 65.3); t_{28} = -3.9; P < 0.001).

Discussion

This study investigated the effects of free-range access on various animal-based welfare indicators (fearfulness, leg health and cleanliness) and the effects of shelter type and weather conditions on free-range use by slow-growing broilers. Compared to similar studies (but under commercial circumstances), the average percentage of chickens outdoors at any given time in the present study was quite high (average around 40%, compared to 4.6 to 11% in other studies; Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). This could indicate that both shelter types (A-frames and SRCW) were adequate for attracting the chickens to the free-range area. However, it is difficult to compare free-range use between studies, as the different studies used different breeds and group sizes which are known to affect free-range use, with a lower proportional free-range use in larger groups (Hegelund et al., 2005; Nielsen et al., 2003). Furthermore, different weather conditions and recording methods may partly explain these differences. It can be questioned if the results from the current study can be extrapolated to commercial-scale farms with larger group sizes, although experience from practice (although with laying hens) learns that SRCW leads to more free-range use with a better distribution of chickens over the free-range area (Boosten, 2015).

It is clear that the SRCW groups used the free-range area significantly more than the AS groups. This is in correspondence with findings of Dal Bosco et al. (2014), who observed that chickens spent more time outdoors if the range area contained trees, and of Dawkins et al. (2003), who observed that chickens preferred trees over grassland when both types of vegetation were located equally far from the house. A possible explanation for this is that the chickens feel safer underneath the trees (Dawkins et al., 2003; Newberry and Shackleton, 1997) because the foliage covers more of the range (almost 100% for the SRCW vs. 8% for the A-frames), although Newberry and Shackleton (1997) did not necessarily find less vigilant behaviour in the presence of cover. Dal Bosco et al. (2014) found fewer predation losses from raptors in free-range areas with tall grass or trees compared to an open area. In the present study no chickens were lost due to raptor attacks. However, it was observed anecdotally that when an airplane or large bird flew over the experimental field, most chickens from the AS groups would go inside the house whilst (more) chickens from the SRCW groups would remain outside. Other reasons for the increased free-range use in the SRCW groups could be that the chickens could not only hide from predators but also from conspecifics, that the biodiversity was greater (Baum et al., 2009; Sage, 1998) with e.g. more insects, or that the soil underneath the willows was better suited for scratching.

The outside chickens in the SRCW groups were more often observed further than 5 m from the house than those from the AS groups. To our knowledge, no other studies have compared between two different shelter types how far the chickens ranged from the stable. Previous studies have compared shelter to no shelter. Zeltner and Hirt (2003) provided laying hens with roofed boxes in the free-range area and observed that these groups ranged farther from the house than groups without shelter. Another study using organic broilers found no effect of providing camouflage nets on the distribution of birds over the free-range area (Rivera-Ferre et al., 2007a). Those nets were only present close to the chicken houses, however. A likely explanation for the observation in the present study that the chickens in the SRCW groups ranged further from the house is that the willows provided an increased sense of safety due to denser and more continuous cover than the cover provided by the A-frames. Other reasons could be that the SRCW provided more shelter against adverse weather compared to the field with A-frames in it, which is confirmed by the differences in e.g. mean wind speed or solar radiation, or that they provided a more interesting environment that stimulated exploration.

Several weather conditions were identified as related to free-range use of the chickens in this study. Rainfall, increasing radiation, and a higher wind speed were related to fewer animals outside. Dawkins et al. (2003) also found a negative relationship between hours of sun and free-range use. Hegelund et al. (2005) found that rainfall, temperatures that increased above 17°C and increasing wind speed were related to less free-range use in laying hens. In the present study, a positive relationship between temperature and free-range use was found, although the effect was quite small (1 °C increase in temperature ~ 0.6% increase in free-range use). These results show that in temperate climates from May to Oct slow-growing broiler chickens prefer to use the free-range area on relatively warm, cloudy days without rainfall or strong winds.

SRCW provided a more dense cover that led to lower wind speeds, radiation and rainfall than in the field with the A-frames (note that measurements were performed next to the A-frames because the weather stations did not fit underneath them). The negative effects of rainfall, increasing radiation and wind speed on free-range use were expected to be smaller in the SRCW than in the AS groups. However, the opposite was true: in the SRCW versus the AS groups, more birds moved inside when it rained or when radiation and wind speed increased. Perhaps the A-frames provided better local protection against the adverse weather when the chickens were actually underneath them. The willows' capacity to shelter may improve as the trees grow and develop. However, under the most prevalent conditions of rainfall, radiation and wind speed recorded in this study, more chickens in total were outside in the SRCW than in the AS groups, indicating either that the trees provide some protection or that the willows provided other attractions that were more important than the weather conditions. The more pronounced effect of adverse weather in the SRCW groups could also be explained by the fact that there were already more birds outside in these groups to begin with, so more birds were 'available' to move inside.

With increasing age, more chickens used the free-range area. This is in agreement with findings of Mirabito and Lubac (2001), Christensen et al. (2003) and Rodriguez-Aurrekoetxea et al. (2014). Age did not affect the number of outside chickens located further than 5 m from their house. Other studies found that broiler chickens ranged further from their house with age (Rodriguez-Aurrekoetxea et al., 2014; Stadig et al., 2014), although the majority of the birds would still stay within 5 m of the house (Stadig et al., 2014). No interaction between age and shelter type was found, which is not in correspondence with Rodriguez-Aurrekoetxea et al. (2014), who found an effect of age and treatment (perches) on distance travelled. This indicates that A-frames and SRCW were equally succesful in facilitating an increasing free-range use with age.

Regarding the TI data, the present study was limited by the lack of individual data for the relationship between fearfulness and free-range use. Although free-range use was not monitored individually, several associations were found that pointed to a relationship between fearfulness and free-range use. The average percentage of chickens outside was not related with the TI duration or the number of inductions in TI trial 1 (before outdoor access). However, a longer mean TI duration at group level in trial 1 was associated with a lower percentage of outside chickens located further than 5 m from the house. A shorter TI duration and a higher number of inductions are associated with less fearful animals (Forkman et al., 2007). It therefore appears that less fearful birds will travel further from the house. To our knowledge, no other studies have assessed fearfulness prior to providing chickens with free-range access.

Chickens from SRCW groups needed more inductions in trial 2 (end of production period) than those from IN groups. This shows a possible relationship between fearfulness and free-range use, where animals that spend more time outdoors receive more stimuli and become therefore less fearful (although there was no difference between IN and AS). This is in agreement with findings of Grigor et al. (1995), Hernandez et al. (2014), and Hartcher et al. (2016) in studies with laying hens. This result, together with the finding that the number of outside birds further than 5 m from the house was negatively associated with TI duration in trial 1, may indicate that fearfulness could affect the distance chickens travel from the house, and that chickens who spend more time outside also become less fearful over time. However, no relationship was found between percentage of animals outside and fearfulness, and when a relationship was found (birds further than 5m from stable; differences between treatment groups), either the duration or the number of inductions was found to be related, but never both. To get more insight into this relationship, data on individual animals would be needed.

Hock dermatitis was shown to be less common in AS and SRCW (tendency) than in IN groups. This type of dermatitis arises predominantly from contact with wet litter (Bassler et al., 2013; Bradshaw et al., 2002). Because birds in the AS and SRCW groups were more active, they perhaps sat down less than the IN birds, thereby decreasing the duration of contact between the hock and the litter. They could also go outside, thus avoiding contact with wet litter. Another explanation could be that the litter in the free-range groups was drier than in the indoor groups, but based on observation (not measurement), it appeared to be the other way around despite replenishing all houses with the same amount of wood shavings). A supporting finding for the first explanation (birds sit less) is that the gait of the AS birds also tended to be better than that of the IN birds, which might have caused these birds to stand or walk more than the indoor ones. Of course, it can also be reasoned that *because* the AS groups walked more than the IN birds they had a better gait and a lower prevalence of hock dermatitis (Falcone et al., 2004; Haye and Simons, 1978; Leterrier et al., 2008).

FPD did not differ among the three treatments. This is not in agreement with Dal Bosco et al. (2014), who found less FPD in birds with access to olive trees than in those with tall grass or an open field and also linked this to the higher level of locomotion in these groups. In the current study, perhaps the poorer litter quality in the mobile houses caused the FPD to be higher in these groups than it would have been if the litter would have been drier. Explanations for the wetter litter in the mobile houses could be suboptimal ventilation, humidity from the underlying soil, or birds bringing wet soil from outside into the houses.

Tibia length was higher in IN than in SRCW birds. This is in correspondence with findings of Foutz et al. (2007), who found a decreased tibia length and cross-sectional area in birds that were forced to exercise. Studies in rats showed that more exercise was related to longer bones with a higher density, which contradicts the findings of the current study (Hart et al., 2001; Newhall et al., 1991; Steinberg and Trueta, 1981). It is possible that the amount of exercise the chickens received in this study was not sufficient to have a relevant impact on bone length, since the difference was only 2 mm. It was also expected that chickens from the AS and SRCW groups had a higher bone strength, which was not found to be the case. An increase in tibia strength in free-range chickens was found by Fanatico et al. (2005b), but in contrast, Wang et al. (2009) found a negative effect of free-range use on tibia strength. Again, the amount of exercise in the current study may not have been high enough to affect bone strength, and it is not certain whether the outdoor birds actually took more steps than the indoor birds. Also, some individuals may have walked much more than others, but because data on free-range use was only gathered on group level, this cannot be linked to bone strength. Alternatively, nutrient intake may be a more important factor in bone strength than exercise; Fanatico et al. (2005b) found no difference in feed intake (**FI**) or body weight (**BW**) between the groups, while Wang et al. (2009) found lower weight gain in the free-range chickens. Perhaps minerals such as calcium in their diet could be the most limiting factor for bone strength.

Conclusions

Providing SRCW in the free-range area instead of AS was beneficial for free-range use, with more chickens recorded outside and using a larger part of the free-range area. The specific features that made the SRCW more attractive to the birds (e.g. shelter from predation and/or adverse weather conditions, stimulation of foraging behaviour, corridor effect) require further study. Wind speed, radiation and rainfall had negative effects on free-range use, indicating that sufficient shelter against adverse weather conditions is required before chickens will go outside. Indications were found that free-range access and use were possibly related to fearfulness. Providing free-range access to slow-growing broiler chickens had some positive effects on their leg health, although for food pad dermatitis it had no effect.

Chapter 3

Preference for shelter type and effects of environmental enrichment

Adapted from:

L.M. Stadig, T.B. Rodenburg, B. Ampe, B. Reubens, and F.A.M. Tuyttens. 2017. Effects of shelter type, early environmental enrichment and weather conditions on free-range behaviour of slow-growing broiler chickens. Animal 11, 1046-1053.

Abstract

Free-range use by broiler chickens is often limited, while better use of the free-range area could benefit animal welfare. Use of free-range areas could be stimulated by more appropriate shelter or environmental enrichment (by decreasing birds' fearfulness). This study aimed to assess the effects of shelter type, early environmental enrichment and weather conditions on free-range use. Three production rounds with 440 slow-growing broiler chickens (Sasso XL451) were carried out. Birds were housed indoors in four groups (2 with males, 2 with females) from days 0-25, during which two of the groups received environmental enrichment. At day 23 birds' fearfulness was assessed with a TI test (n = 100). At day 25 all birds were moved (in mixed-sex groups) to mobile houses, and provided with free-range access from day 28 onwards. Each group could access a range consisting for 50% of grassland with AS (21 wooden A-frames) and for 50% of SRCW (dense vegetation). Free-range use was recorded by live observations at 0900 h, 1300 h and 1700 h for 15-21 days between days 28 and 63. For each bird observed outside the shelter type (AS or SRCW), distance from the house (0-2 m, 2-5 m, >5 m) and its behaviour (only rounds 2 and 3) were recorded. Weather conditions were recorded by four weather stations. On average, 27.1% of the birds were observed outside at any given moment of observation. Early environmental enrichment did not decrease fearfulness as measured by the TI test. It only had a minor effect on the percentage of birds outside (0.4% more birds outside). At all distances from the house, SRCW was preferred over AS. In AS, areas closer to the house were preferred over farther ones, in SRCW this was less pronounced. Free-range use increased with age and temperature and decreased with wind speed. In AS, rainfall and decreasing solar radiation were related to finding more birds outside, while the opposite was true in SRCW. Behaviour of the birds depended on shelter type, distance from the house, early environmental enrichment, time of day and age. Chickens ranged more and farther in SRCW, possibly because this provided a greater sense of safety because of the amount of cover and/or better protection against adverse weather conditions. These results indicate that SRCW with willow is a more appropriate shelter for slow-growing broiler chickens than A-frames.

Introduction

Free-range use in broiler chickens is often limited, with only 5-11% of birds being outside at any given time (Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). If the chickens would go outside more often and distribute themselves over the field more evenly as opposed to staying close to the house, their welfare could benefit in several ways.

They would have more opportunities to perform highly motivated natural behaviours; more foraging opportunities may be available farther from the house due to more (edible) vegetation, and better distribution over the range would give them more space for dust bathing and other behaviours. Better distribution over the range could prevent point pollution close to the house, and such decreased stocking density may also lead to lower nematode infection levels (Sherwin et al., 2013).

A more optimal use of the free-range area could be accomplished by providing appropriate shelter (Dawkins et al., 2003; Dal Bosco et al., 2014). The preference of broiler chickens for different shelter types, however, remains unclear. One previous study showed that when birds had the choice, they preferred areas with vertical panels over grassland (Stadig et al., 2014), but when comparing groups with panels to groups with grassland, no differences in free-range use were observed (Rodriguez-Aurrekoetxea et al., 2014). Perhaps the panels in the latter study did not cover a large enough part of the free-range area to have a profound impact, or they did not have the desired characteristics (e.g. protection against solar radiation or aerial predators). Studies comparing free-range use of chickens with different shelter types indicate that those with access to trees spend considerably more time outside in comparison with access to grassland or artificial shelter, but the actual preference of the chickens could not be tested in these setups (Dal Bosco et al., 2014; Chapter 2 of this thesis). Only one observational study testing the preference for shelter type showed that birds preferred trees over grassland when these were located at a similar distance from the house (Dawkins et al., 2003). SRCW could be an interesting type of vegetation suitable for free-range areas for chickens. SRCW is planted at very high density (15,000 trees/ha), can be harvested every 3 years, and the wood chips can be used for heat or energy production or as litter. The dense vegetation may be attractive to the birds as it can provide protection against adverse weather conditions and aerial predators, and the alleged increase in biodiversity (Sage, 1998; Baum et al., 2009) may increase foraging behaviour.

Another factor that might play a role in free-range use is fearfulness. Studies with laying hens have shown that decreased fearfulness is linked to increased free-range use (Grigor et al., 1995; Hartcher et al., 2016). In addition, it has been shown that early environmental enrichment (coloured drawings on the wall, brightly coloured manipulable objects) can reduce fearfulness in laying hen chicks (Jones and Waddington, 1992). However, this has not yet been studied in broiler chickens. Stadig et al. (Chapter 2 of this thesis) found some relations between fearfulness of slow-growing broilers at group level as measured by a TI test (test of fearfulness (Jones, 1986)) and free-range use (a shorter pre-outdoor-access TI duration was associated with more chickens >5 m from the house, and the % of animals needing more than one induction to induce TI was higher for chickens from groups that used the free-range area more). However, these findings were not conclusive, perhaps due to little variation between the groups, as there was no treatment aimed at reducing or augmenting fearfulness. Additionally, the interactive effects of early environmental enrichment and fearfulness on free-range use have not yet been studied within a single experiment.

In addition to shelter and fearfulness, weather conditions are also known to influence free-range use. It has been found that in temperate climates rainfall, high wind speeds, and high solar radiation have a negative impact on the number of birds outside (Chapter 2 of this thesis), while an increase in temperature has a positive effect (Dawkins et al., 2003; Rodriguez-Aurrekoetxea et al., 2014; Chapter 2 of this thesis). However, the impact of these weather conditions may differ depending on shelter type: for example, artificial roofs may protect better against solar radiation, while SRCW may provide better protection against strong wind.

The aims of this study were to test the following hypotheses: 1) that early environmental enrichment would decrease fearfulness and thereby increase free-range use, 2) that chickens would prefer SRCW over grassland with AS, 3) that enrichment would lead to more foraging behaviour (because this was stimulated by the enrichment), 4) that behaviours would differ between shelter types and distance from the house (e.g. more sitting behaviour in the SRCW because it provides a better sense of safety than AS, and more foraging behaviour far from the house due to more vegetation), and 5) that rainfall, strong winds and strong solar radiation would be related with a decrease in free-range use, but that these aspects would mainly affect the number of chickens in AS because SRCW provides better protection.

Materials and Methods

Animals and housing

From March to October 2015, three production rounds were completed. Each round included 440 slow-growing mixed-sex broiler chickens (Sasso XL451). The Sasso hybrid was chosen because it is the most common hybrid in organic broiler production in Belgium, where organic broilers are the predominant type of broilers that get free-range access (Tuyttens et al., 2014). The chickens were housed indoors from day 0 until day 25, in four groups of 110 birds (6 m^2

per pen; two pens with males and two with females). They received feed and water *ad libitum* (starter wk 0-3, grower wk 4-7, finisher wk 8-10). The feed was produced by ILVO (see Chapter 5 for the exact composition). Half of the four groups (i.e. one male and one female group) received environmental enrichment until day 25; the other two did not. The enrichment consisted of a hay bale (replaced twice per week), white strings (on the floor, attached to the walls and hanging from the ceiling), daily provision of grain which was not put in the feeders but distributed over the litter (wood shavings), and live mealworms (ca. 200 g / group / day). These enrichments were chosen to encourage exploration and foraging behaviour.

At day 25, chickens were moved to four mobile houses (McGregor Polytunnels Ltd., Ropley, UK; Figure 3.1). Males and females were mixed upon moving to achieve a male:female ratio of 50:50 in each group. Birds from enriched groups were placed together, as were birds from unenriched groups. The mobile houses were located on a 100 m x 100 m field (Figure 3.2) and were repositioned along the AS/SRCW boundary in between rounds to increase use of the entire free-range area and minimise point pollution. They measured 4.1 m x 4.25 m and their floors were covered with wood shavings. Each house contained two bell drinkers and two feeders. They were isolated with fibreglass and ventilated naturally through openings in the two doors. From day 28 onwards, birds were given free-range access every day from the morning (between 0700 h and 0900 h) until sunset. They had access to a field (1600 m^2), half of which was grassland with AS (21 wooden A-frames; L x W: 2.5 m x 1.25 m; height at the centre: 1.5 m; Figure 3.1) and the other half was SRCW (Figure 3.1) planted with willow clones (Tora (S. schwerinii), Klara ((S. burjatica x S. viminalis) x S. burjatica) and Tordis (S. schwerinii x S. viminalis)). The trees had been planted in the spring of 2013 at high density (15,000 trees/ha) in accordance with common practice (75 cm between single rows, 150 cm between double rows, 60 cm between trees in each row). During the periods in which the chickens had outdoor access, the trees had leaves (although they only started to grow at the time that the first round went outside) and were on average 4.32 m tall. At day 70, all birds were weighed in groups (males and females separately). At day 72, all chickens were commercially slaughtered and processed. All animal procedures were approved by the Ethics Committee of the Institute for Agricultural and Fisheries Research (ILVO, Merelbeke, Belgium).

Fearfulness

At day 23, a TI test was performed on 25 randomly selected chickens per group. This is the reason why the chickens were initially housed in single-sex groups; we wanted to have equal numbers of males and females for the TI tests (because some aspects of the TI test may differ between sexes (Jones and Faure, 1981)), and at day 23 it was not yet possible to differentiate between sexes based on e.g. comb size and colour. During this test, the chicken was placed on its back in a U-shaped crib, restrained for 15 s and then released. If the chicken righted itself within 10 s, another induction was done, with a maximum of three inductions. If the induction was successful, the duration until righting and the number of inductions were recorded. Maximum duration of the test was 5 minutes; in this case the bird was allocated a duration of 300 s.

Free-range use and behaviour

Free-range use was recorded by visual observations during 15 days in round 1, 21 days in round 2, and 18 days in round 3, starting on day 28 and ending on day 63. The observations were always done at 0900 h, 1300 h and 1700 h. The number of animals outdoors was counted once per hour, and for every chicken that was outdoors it was recorded which part of the field (AS or SRCW) it was on and how far it was away from the house (0-2 m, 2-5 m, >5 m). In addition, in rounds 2 and 3, the behaviour of each chicken at that moment was scored: the possibilities were standing, walking, running, sitting/lying, preening, dust bathing, foraging/eating and social behaviour (interaction with other birds). When scoring, preening, dust bathing, foraging/eating and social behaviour had priority over standing, walking, running and sitting/lying. The scoring of the behaviour was done according to the birds' location: first the birds within 0-2 m from the house were scored, then those within 2-5 m, and finally those >5 m from the house. Running was rarely observed and therefore, for the analysis walking and running were grouped together as 'locomotion'. Preening, dust bathing and social behaviour were also rare, and were grouped together as 'other' behaviours.

Figure 3.1 Left: free-range area with wooden A-frames. Middle: free-range area with short rotation coppice willows. Right: mobile house.

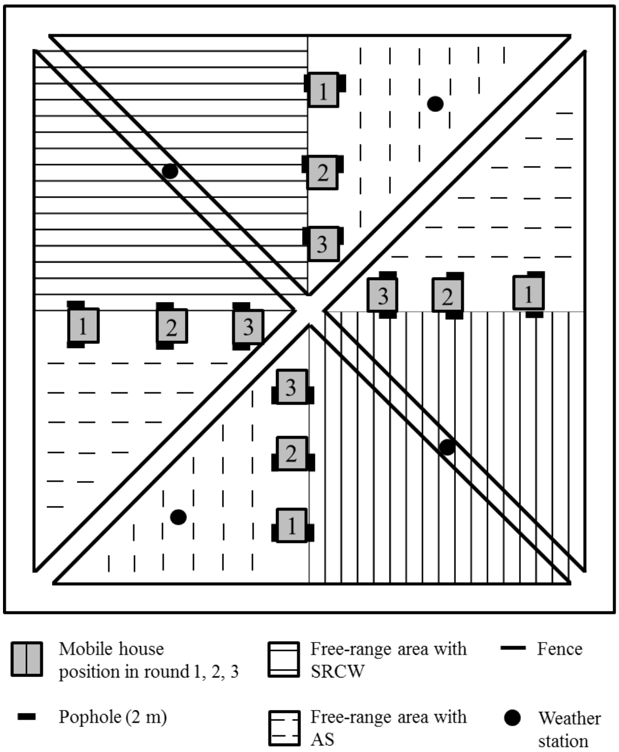


Figure 3.2 Top view of the experimental site (100 m x 100 m (outer edges)) with short rotation coppice willows (SRCW) and grassland with artificial shelter (AS).

Weather conditions

In total four weather stations were placed on the field: two in AS (they did not fit underneath the A-frames and were therefore placed in the open area next to a frame) and two in SRCW (Figure 3.2). The weather stations were equipped with a thermometer and humidity meter (CS215, Campbell Scientific, Logan, UT, USA), a precipitation meter (ARG100 Tipping Bucket, Campbell Scientific), a pyranometer (CS300, Apogee Instruments, Logan, UT, USA), a wind speed meter (03002 Wind Sentry Set, R.M. Young, Traverse City, MI, USA), and a data logger (CR200, Campbell Scientific). They made recordings every 15 minutes of the following five parameters: temperature (°C), relative humidity (%), solar radiation (kW/m^2), rainfall (mm/15 min) and wind speed (m/s). For the analysis, the average of the values recorded by all four weather stations was used from the point in time closest to the observation time.

Data analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Data are presented as LS means ± standard errors unless stated otherwise. Continuous variables were considered sufficiently normally distributed based on the graphical evaluation (histogram and QQ plot) of the residuals. Statistical significance was evaluated at $P < 0.05$. Non-significant factors were removed from the final models. In case of post-hoc pairwise comparisons, the Tukey-Kramer adjustment for multiple comparisons was used at a total significance level of

0.05. The effect of enrichment on TI duration and number of inductions was tested using a generalised linear mixed regression model. TI duration was log-transformed. Fixed factors were enrichment (yes/no) and BW (per sex). Pen within production round was included as random effect. Data are shown as back-transformed LS means and their back-transformed confidence intervals (**CI**).

Data on free-range use and behaviour were analysed using a GLM Poisson regression model, with a first-order autoregressive covariance-structure to correct for multiple observations over time within the same house. Early enrichment (yes vs. no), shelter type (AS versus SRCW), distance from the house (<2 m, 2-5 m, >5 m), time of day (0900 h, 1300 h or 1700 h), age, rainfall, temperature, wind speed, relative humidity, solar radiation and their relevant interactions were included in the model for free-range use (% chickens outside; log-transformed) as fixed factors, but removed again if not significant. In the model for behaviours (expressed as percentage of the birds outside performing that behaviour) fixed factors were early enrichment, shelter type, distance from the house, time of day, age, and their relevant interactions.

Results

Production

The average mortality per round was 2.5 ± 0.3% (mean ± standard deviation). The average weight of male birds at day 70 was 3.0 ± 0.2 kg, that of females was 2.3 ± 0.2 kg.

Fearfulness

In 300 TI tests, TI could not be induced in 13 chickens, and 16 chickens reached the maximum duration of 300 s. Enrichment had no effect on fearfulness as measured by the TI duration (t_{286} = -0.57; P = 0.571). TI duration was however related to the weight of the bird, with a longer duration in heavier birds, both in males (t_{142} = -1.29; P = 0.023) and in females (t_{143} = 6.41; P = 0.012). For example, a male bird in an enriched group weighing 300 g (minimum recorded weight) would have on average a TI duration of 22 s, compared to 88 s for a bird weighing 780 g (maximum recorded weight).

Free-range use

On average, 27.1% (95% CI: 24.8 – 29.7) of the birds were observed outside at any given moment of observation. Table 3.1 gives an overview of all factors that were related to the number of birds outside. Although enrichment did not affect birds' fearfulness, it did have a small, positive effect on the number of birds outside as compared to the groups without enrichment (0.4% more birds outside, $t_6 = -2.49$; P = 0.047). At all distances from the house, a markedly higher number of birds were observed in SRCW compared to AS (all P < 0.001; Figure 3.3). In AS more birds were within 0-2 m than within 2-5 m ($t_{14} = -5.92$; P < 0.001) or >5 m ($t_{14} = 8.01$; P < 0.001) from the house, and more birds tended to be within 2-5 m than >5 m ($t_{14} = 3.07$; P = 0.072; Figure 3.3). In SRCW, the percentage of birds within 0-2 m of the house did not differ from that within 2-5 m ($t_{14} = 2.43$; P = 0.211) or >5 m ($t_{14} = 2.04$;

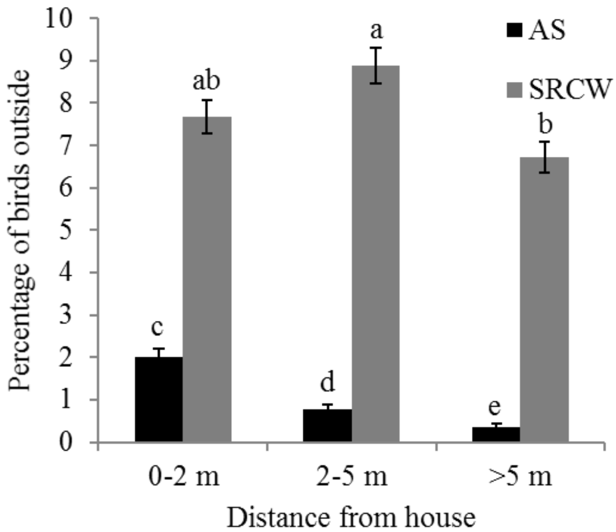


Figure 3.3 Percentage of broiler chickens outside in AS and SRCW, according to their distance from the house. Bars without a common superscript differ significantly (P < 0.05). AS = grassland with artificial shelter, SRCW = short rotation coppice willows.

Table 3.1 Factors related to the number of broiler chickens outside at a given moment of observation.

Factor	DF	F Value	P value
Enrichment	6	5.75	0.047
Distance from house	14	32.86	< 0.001
Shelter	7	98.41	< 0.001
Shelter x Distance	14	42.42	< 0.001
Age	2303	57.66	< 0.001
Age x Distance	2303	17.33	< 0.001
Time of day	14	5.18	0.021
Temperature	2303	4.37	0.037
Rain x Shelter	2303	14.26	< 0.001
Solar radiation x Shelter	2303	6.43	0.002
Wind speed	2303	4.88	0.027

Table 3.2 Mean, minimum and maximum values of all recorded weather variables per shelter type.

Variable	AS			SRCW		
	Mean	Min	Max	Mean	Min	Max
Wind speed (m/s)	0.9	0	5.5	0.2	0	2.7
Solar radiation (kW/m^2)	0.15	0	0.97	0.05	0	0.87
Temperature (°C)	14.8	-1.9	33.6	15.1	-1.2	33.1
Relative humidity (%)	84.9	36.0	100	88.0	38.1	100
Rain (mm/15 min)	0.02	0	10.6	0.01	0	8.2

AS = grassland with artificial shelter; SRCW = short rotation coppice willows

P = 0.368) from the house, but the percentage within 2-5 m was higher than that at >5 m (t_{14} = 4.04; P = 0.012; Figure 3.3).

Free-range use increased with age of the chickens. This effect was more pronounced in areas farther from the house (F_{2303} = 17.33; P < 0.001; Figure 3.4). For example, the mean percentages of birds at 0-2 m, 2-5 m and >5 m, respectively, were 8.5% (95% CI: 6.8 – 10.7), 6.9% (95% CI: 5.5 – 8.7) and 3.9% (95% CI: 3.0 – 5.2) at day 28, and 11.6% (95% CI: 8.6 – 15.7), 16.4% (95% CI: 12.5 – 21.7) and 19.9% (95% CI: 15.0 – 26.5) at day 63. The mean percentage of birds outside was related to time of day (raw means ± st. dev.: 32.1 ± 17.1% in the morning vs. 22.6 ± 16.5% in the afternoon; F_{14} = 5.18; P = 0.021) with birds outside at midday (25.7 ± 17.6%) being intermediate and not different from that observed either morning or afternoon.

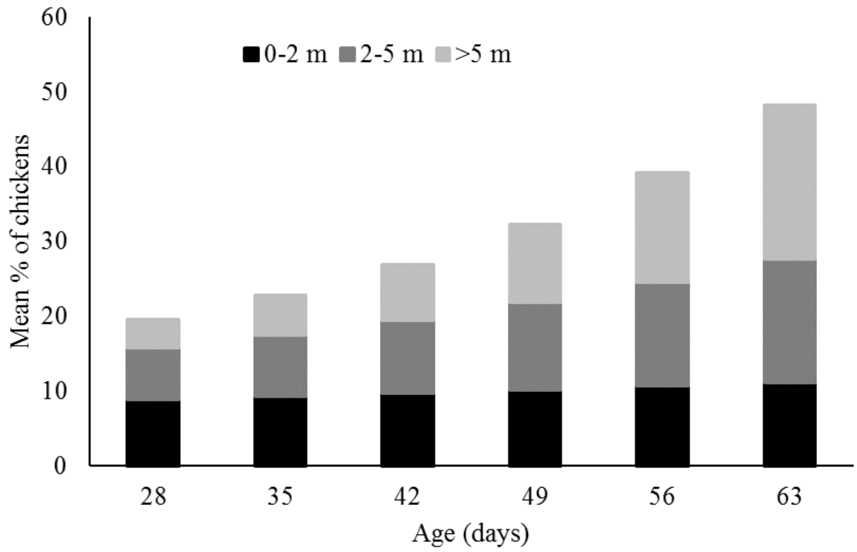


Figure 3.4 Mean percentage of broiler chickens outside at different ages, per distance from the house (0-2 m, 2-5 m, >5 m).

Weather conditions had an effect on free-range use (for an overview of the weather conditions during the experiment see Table 3.2). Free-range use increased with temperature (F_{2303} = 4.37; P = 0.037), and decreased with wind speed (F_{2303} = 4.88; P = 0.027). In AS, rainfall and decreasing solar radiation were related to more birds outside, while the opposite was true in SRCW (F_{2303} = 14.26; P < 0.001 and F_{2303} = 6.43; P = 0.002). For example, at a temperature of 15 °C, 23.6% (95% CI: 21.2 – 28.9) of the birds would be outside, whilst at 30 °C this would be 33.5% (95% CI: 25.2 – 44.5). In AS, when it was not raining a mean percentage of 3.0% (95% CI: 2.3 – 3.7) of the birds was outside, as compared to 5.5% (95% CI: 4.0 – 10.6) when it was raining. For SRCW this was 24.8% (95% CI: 23.6 – 27.8) when it did not rain vs. 11.9% (95% CI: 8.1 – 16.8) when it rained. In AS, at a radiation of 0.1 kW/m^2 mean percentage of birds outside was 3.7% (95% CI: 2.8 – 5.1), vs. 2.3% (95% CI: 1.5 – 3.2) at 0.4 kW/m^2 °C. In SRCW, this was 21.9% (95% CI: 19.0 – 25.1) at 0.1 kW/m^2 and 25.9% (95% CI: 21.6 – 31.0) at 0.4 kW/m^2.

Free-range behaviour

Occurrence of several behaviours was related to shelter type, distance from the house, time of day, and the age of the birds (Table 3.3). Occurrence of the behaviours was expressed as the percentage of the total number of birds that were outside. We chose not to use absolute numbers in order to avoid confounding effects of factors such as shelter type: the fact that birds preferred the SRCW would mean the chance any behaviour was more common in the SRCW would be higher than in the AS. On the other hand, using percentages can give a distorted picture of the absolute occurrence of these behaviours. Therefore, when interpreting these results, one should keep in mind that the number of birds in AS was considerably lower than in SRCW.

Table 3.3 gives an overview of the factors affecting behaviours that the birds performed in the free-range area. In groups that had received early environmental enrichment, a lower percentage of the birds was standing (F_{1197} = 30.89; P < 0.001) and a higher percentage was sitting (F_6 = 6.00; P = 0.050) as compared to the groups without enrichment. The estimated percentage of outside birds observed standing was higher in SRCW than in AS (F_{1197} = 24.22; P < 0.001), decreased with distance from the house (F_{1197} = 179.73; P < 0.001), decreased with age (F_{1197} = 34.29; P < 0.001) and was higher in the morning than later on the day (F_{1197} = 24.22; P < 0.001). Locomotion (walking + running) did not differ between shelter type or time of day, but occurred more often in birds furthest from the house (F_{14} = 3.86; P = 0.046) and decreased with age (F_{1194} = 24.69; P < 0.001). A higher percentage of birds in SRCW were sitting when compared to those in AS ($F_7 = 42.08$; $P < 0.001$). Sitting occurred less in birds furthest from the house ($F_{14} = 46.99$; $P < 0.001$), was observed more often at midday than in the morning or afternoon ($F_{14} = 24.35$; $P < 0.001$), and increased with age ($F_{1191} = 126.44$; $P < 0.001$). Foraging was performed by a higher percentage of birds in AS compared to SRCW ($F_7 = 52.41$; $P < 0.001$), by birds farther from the house ($F_{14} = 100.48$; $P < 0.001$), and in the morning as compared to midday and afternoon ($F_{14} = 42.87$; $P < 0.001$), and decreased with age (F_{1191}; 42.43; $P < 0.001$). Dust bathing, preening and social behaviour (grouped as 'other' behaviours) were not affected by shelter type or age, but occurred relatively more often close to the house ($F_{14} = 11.71$; $P = 0.001$), and later in the day ($F_{14} = 14.38$; $P < 0.001$).

Table 3.3 Factors affecting the occurrence of behaviours of broiler chickens in the free-range area at a given moment of observation (% of total number of birds outside).

Factor		Average number of birds outside	Standing	Locomotion[1]	Sitting and lying	Foraging	Other[2]
Early enrichment	Yes	30.5	5.9[b]	11.0	27.2[a]	3.5	4.0
	No	27.4	8.1[a]	10.7	23.3[b]	4.0	3.8
Shelter	AS	4.1	5.5[b]	13.7	18.1[b]	50.2[a]	4.7
	SRCW	25.0	8.5[a]	10.5	35.7[a]	27.8[b]	3.8
Distance	0-2 m	11.5	15.2[a]	9.1[b]	30.7[a]	25.1[c]	4.6[a]
	2-5 m	9.4	5.6[b]	10.6[ab]	30.5[a]	32.3[b]	6.7[a]
	>5 m	8.0	3.7[c]	13.3[a]	17.6[b]	64.1[a]	2.5[b]
Time of day	Morning	35.0	9.0[a]	12.0	15.8[c]	54.6[a]	2.4[a]
	Midday	32.0	6.4[b]	11.2	27.5[a]	28.8[bc]	4.2[a]
	Afternoon	27.2	5.6[b]	13.1	22.1[b]	33.0[c]	7.5[b]
Age[3]			decrease	decrease	increase	decrease	no difference

AS = grassland with artificial shelter; SRCW = short rotation coppice willows

[1] *Locomotion includes walking and running*

[2] *Other includes dust bathing, preening and social behaviour*

[3] *Indicates whether behaviours increased or decreased with age*

[a-c] *Means per factor within a column without a common superscript differ significantly (P<0.05)*

Discussion

In this study we aimed to reduce fearfulness by providing birds with environmental enrichment at an early age. However, enrichment had no effect on fearfulness as measured by a TI test. TI duration was positively correlated with BW. Perhaps it was physically more difficult for heavier birds to right themselves. At this young age (day 23), and therefore low weight, that may not yet seem to play a role, but Bizeray et al. (2000) already found differences in chickens' activity related to BW from day 15 onwards. Early-life enrichment did have a small positive effect on the mean number of chickens outside, so perhaps it influenced the birds in another way than via fearfulness. Nevertheless, this effect was small and its relevance can be questioned. Enrichment did not affect the distance (0-2, 2-5 or >5 m) the chickens ranged from the house or the shelter type they preferred. The period of enrichment-provision was possibly too short (4 weeks), although Jones and Waddington (1992) did find an effect of providing enrichment (manipulable, brightly coloured objects) to layer chicks from days 0-20 on fearfulness as measured by TI, OF, novel object and emergence tests. Alternatively, the enrichment was insufficient (either in quantity or quality) to have a strong effect. The enrichment did influence standing (shown less) and sitting (shown more). The differences in standing and sitting are not in accordance with other studies on early environmental enrichment: Jones and Waddington (1992) found that chicks from enriched pens pecked more at the environment, but found no other differences in home-pen behaviour, and Jones (1982) observed less immobility in an OF test in chicks from enriched pens. The reason for the differences in standing and sitting behaviour in this study remains unclear, but the effect sizes were small and may not be relevant.

Free-range use was overall higher than in other studies with broiler chickens (Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). The reason for this is unclear, but could be related for instance with a smaller group size (Hegelund et al., 2005), a different breed (Nielsen et al., 2003), or a better free-range design (Dawkins et al., 2003). Many factors had an effect on how chickens used the free-range area. The chickens showed a clear preference for SRCW over AS. There are several possible explanations for this: the SRCW's dense vegetation likely provided a greater sense of safety (it was anecdotally observed that more chickens went inside the house when an airplane or large bird flew over the field if they were in AS compared to SRCW), the SRCW provided protection against adverse weather conditions (average wind speed and solar radiation were lower in SRCW than in open areas in AS), and an increased biodiversity in the SRCW is likely to have provided an attractive foraging environment (not measured in this study). Regarding the weather conditions, the number of birds in SRCW indeed increased with increasing solar radiation, whereas the number of birds in AS decreased, indicating that SRCW provided more shade than AS. However, when it rained the number of birds in SRCW decreased, while the number in AS increased. The increase of birds in AS was numerically very small, and the absolute number in SRCW was always higher, indicating that AS did not provide sufficient protection against rain. Regardless of shelter type, free-range use decreased with decreasing temperatures and increasing wind speeds. This indicates that AS and SRCW provided similar protection against these factors, in accordance with findings in Chapter 2 of this thesis, Dawkins et al. (2003) and Rodriguez-Aurrekoetxea et al. (2014).

Shelter type not only affected the distribution of birds over the range, but also the behaviours the birds performed. A higher percentage of the chickens in AS foraged as compared to SRCW, but the number of birds in SRCW was considerably higher than in AS, so the absolute number of birds foraging was actually higher in SRCW than in AS. This could be because foraging is a highly motivated behaviour in chickens (Weeks and Nicol, 2006) and therefore occurs frequently regardless of the environment, but it could also indicate that the vegetation in SRCW was more attractive for foraging. In SRCW both standing and sitting were proportionately more common than in AS. A possible explanation is that birds felt safer in SRCW, and were therefore more likely to sit down, which could have caused them to be less mobile and therefore more vulnerable to predators. The roots of the trees could also be suitable dust bathing spots, after which the chickens remain sitting at that spot. Dal Bosco et al. (2014) also found differences in behaviours between birds on pasture vs. with tall grass vs. with olive trees, such as more lying down in pasture and more locomotion under the trees. However, they always observed the same number of birds per shelter type, and the absence of the confounding effect of total number of birds outside could account for differences with our study. Rodriguez-Aurrekoetxea et al. (2015) found no effect of shelter (panels) in the free-range area on behaviour, possibly due to their light weight, which may have made them unstable and therefore not attractive enough (Rodriguez-Aurrekoetxea et al., 2015), or perhaps there were not enough shelters placed outdoors (nine 0.5 m x 0.5 m panels for 1300 birds).

Chickens preferred the areas closer to the house over those farther away, especially in AS, which is in accordance with findings of Mirabito and Lubac (2001) and Dawkins et al. (2003). However, in SRCW there was no difference between 0-2 m and 2-5 m from the house. Perhaps this was because the chickens had to cross a small patch of grassland (1 m) in front of the house to reach the willows, giving them more incentive to go farther from the house, or because SRCW was more suitable to encourage the birds to range farther from the house. The chickens were more often observed to be standing or sitting close to the house as compared to farther away. This could be due to an increased feeling of safety when closer to the house, or because there was no need to range farther from the house to sit down, whilst for foraging, it was necessary to traverse greater distances to access suitable foraging places.

Free-range use was highest in the morning, which corresponds with findings of Dawkins et al. (2003) and Jones et al. (2007). These studies however also found an increase in free-range use again in the late afternoon / early evening. In our study this was not observed; perhaps the observations in the afternoon were still too early (2-5 hours before sunset) to observe an increase in free-range use at the end of the day. Behaviours were also affected by time of day: sitting was most often observed at midday, while more active behaviours such as standing and foraging were observed most in the morning. This suggests that at the times that the most birds are outside, they are also most active.

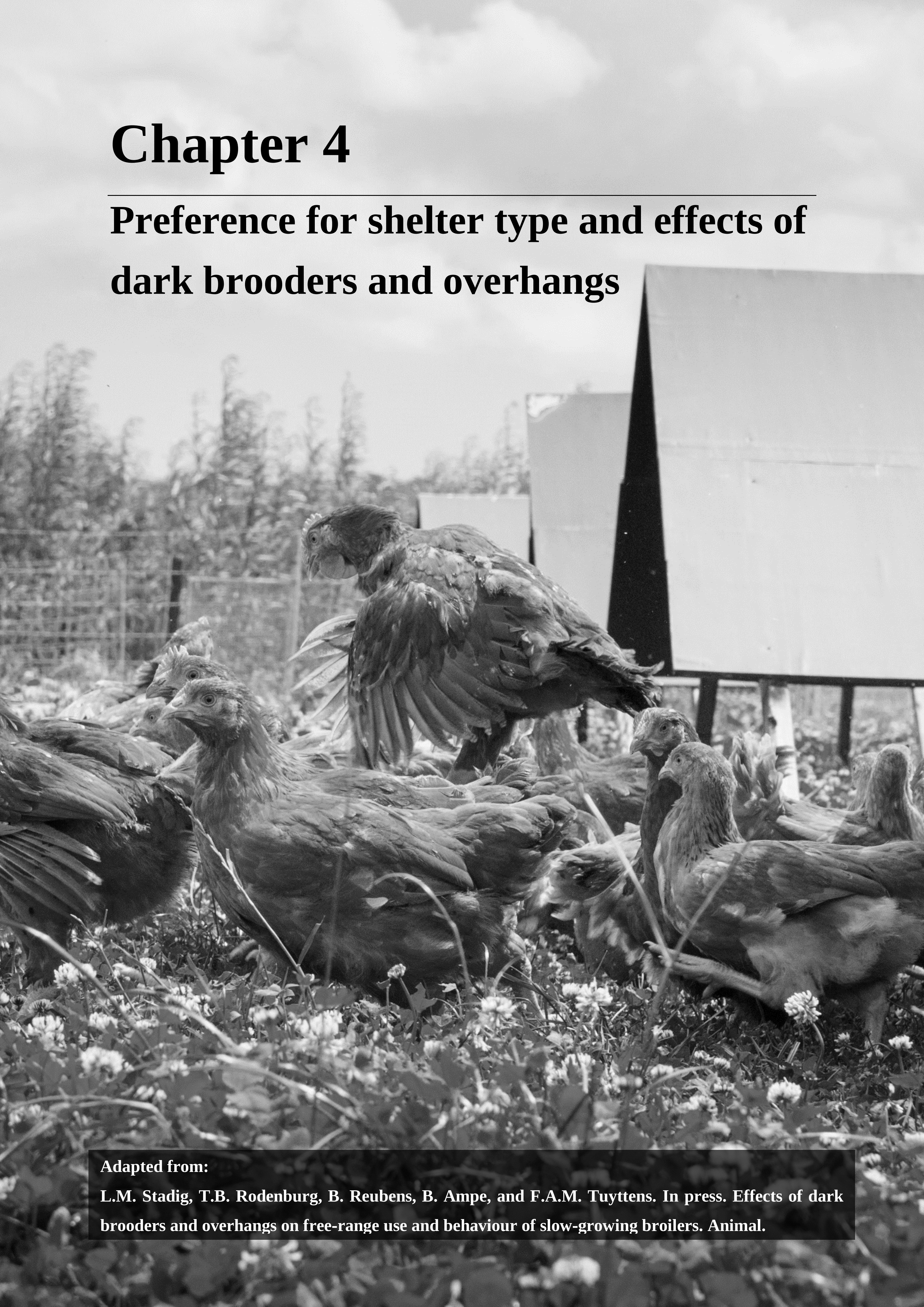
Free-range use increased with age, and this effect was more pronounced at distances farther from the house. The finding that free-range use increased with age corresponds with findings of Mirabito and Lubac (2001) and Rodriguez-Aurrekoetxea et al. (2014), and could indicate that chickens may need time to get used to free-range access. The observation that older birds ventured farther from the house could be because (edible) vegetation near the house became depleted over time, because they learned it was safe to go farther, or because they were less prone to predation due to their larger body size. Age was also related with the behaviours the birds showed outside, with sitting being observed more often over time, and standing, locomotion and foraging less. This could be due to the increasing weight of the birds, which made them less mobile (Bokkers and Koene, 2003). Rodriguez-Aurrekoetxea et al. (2015) found no effect of age on slow-growing broilers' behaviour, but they only used females in their experiment, which are less heavy at slaughter age, so weight would have less influence on their activity.

Conclusions

Early environmental enrichment had no effect on birds' fearfulness and no profound effect on free-range use. Birds showed a strong preference of SRCW over AS in the free-range area, and they also ranged farther from the house in SRCW, indicating that this shelter type was more suitable, e.g. because of protection against adverse weather conditions. Exactly which characteristics of SRCW caused this difference needs further investigation. Chickens showed other behaviours in SRCW than in AS, such as relatively more sitting and lying. This study's results indicate that SRCW is a more appropriate shelter type for slow-growing broiler chickens than A-frames.

Chapter 4

Preference for shelter type and effects of dark brooders and overhangs



Adapted from:

L.M. Stadig, T.B. Rodenburg, B. Reubens, B. Ampe, and F.A.M. Tuyttens. In press. Effects of dark brooders and overhangs on free-range use and behaviour of slow-growing broilers. Animal.

Abstract

Broiler chickens often make limited use of the free-range area. Range use is influenced by type of shelter available. Range use may possibly be improved by a more gradual transition from the house to the range and by using dark brooders (secluded warm, dark areas in the home pen) that mimic aspects of a broody hen and possibly reduce fearfulness. The aim of this study was to assess effects of dark brooders on fearfulness, free-range use and behaviour later in life. Another aim was to test the chickens' preference for shelter type and the effects of overhangs outside of the pop holes to provide a gradual transition to the range. Three production rounds, each with 440 Sasso broiler chickens (110 per group), were completed. Chicks were housed indoors from day 0-25; per round, two groups had access to a dark brooder, while the other two groups had conventional infrared lamps. Fearfulness was assessed by OF and TI tests on days 22-24 on 25 chicks per group per round. Birds were then moved to four mobile houses from which they could access both grassland with AS and SRCW. Two of the houses had overhangs extending from the pop holes; these were switched between the four houses weekly. Free-range use and behaviour were observed three times daily from Monday to Friday. Dark brooders did not affect results from the OF or TI test, except for jumps in the OF test which tended to occur more often in non-brooded chicks. Neither dark brooders (34.9% without vs. 31.7% with brooder) nor overhangs (32.5% without vs. 34.1% with overhangs) influenced the percentage of chickens outside. Chickens showed a clear preference for SRCW, range use increased over time in SRCW, and more birds ranged farther from the house in SRCW. Behaviours of chickens observed outside were mainly influenced by shelter type, broiler age and distance from the house. Locomotion tended to occur more in the presence of overhangs. Overall, these results could not confirm the hypothesis that dark brooders would decrease fearfulness and thereby increase free-range use. Overhangs also did not improve free-range use, and neither brooders nor overhangs had considerable impact on behaviour of chickens outside. Chickens clearly preferred dense natural vegetation over artificial shelter and ranged farther in it, indicating that this type of shelter is more suitable for slow-growing free-range broilers.

Introduction

Limited use of the free-range area is common in broiler chickens with outdoor access; on average only a small part of the flock is found outside at any given moment, and the majority of the birds remain close to the chicken house (Dawkins et al., 2003; Jones et al., 2007). Improved use of the range could benefit broiler welfare as a result of factors including increased space per animal, better air quality outside compared to inside, and access to more substrates for natural behaviours such as sand for dust bathing and natural vegetation for foraging. A better distribution of the flock over the range would also benefit the environment; a lower bird density close to the chicken house would lead to a reduction in point pollution of N and P (Dekker et al., 2012).

Limited use of the free range has multifactorial causes. A prior study has shown that shelter plays an important role: if birds had access to both SRCW and grassland with AS (wooden A-frames), they showed a clear preference for SRCW (Chapter 3 of this thesis). Another factor that may increase free-range use is a more gradual transition from inside the house to the range, e.g. by providing some kind of shelter just outside the house. A study with 33 flocks of laying hens showed that a higher light intensity in the house was associated with a higher percentage of hens using the range (Gilani et al., 2014). Dekker et al. (2012) suggested that the higher free-range use in laying hens with transparent curtains between the house, winter garden and outdoor run was due to the smaller differences in light intensity between these areas in comparison to farms with opaque curtains. An alternative for these transparent curtains may be overhangs in front of the pop holes. These give the birds the opportunity to go outside without immediately being exposed to factors such as bright light, precipitation and aerial predators. After this transition they may feel more confident to proceed into the range.

Free-range use may be influenced by fearfulness. In laying hens, there are indications that increased fearfulness is related to lower free-range use (Campbell et al., 2016a; Grigor et al., 1995; Hartcher et al., 2016). Stadig et al. (Chapter 2 of this thesis) found some relationships between fearfulness and free-range use in broiler chickens, although these were tested on group level and not on individual level. If a relation between fearfulness and free-range use in broiler chickens does exist, decreasing fearfulness could lead to increased free-range use.

Use of dark brooders during the rearing phase (before free-range access is provided) may reduce fearfulness. Dark brooders in this study were elevated, heated wooden panels curtained off by black rubber curtains with fringed bottoms. Dark brooders give chicks access to a warm, secluded, dark area and give chicks the choice between being in an active group or inactive group (Jensen et al., 2006). In laying hens, provision of dark brooders is related to a reduction in feather pecking and cannibalism (Gilani et al., 2012; Jensen et al., 2006), which are known to be associated with fearfulness (Bolhuis et al., 2009; Jones et al., 1995; Keeling and Jensen, 1995; Rodenburg et al., 2013). Indications for reduced fearfulness in laying hens with access to dark brooders have been found by Gilani et al. (2012) and Riber and Guzman (2016). One study showed that dark-brooded laying hen pullets engaged less often in locomotion and fleeing and foraged more than control hens at day 42 (Riber and Guzman, 2016). Laying hen pullets reared with either a mother hen or a dark brooder showed longer bouts of activity at young age, while the total time budget did not differ between brooded and non-brooded chicks (Riber et al., 2007; Wauters et al., 2002). Dark brooders could partially mimic a broody hen, providing a safe place from where the chicks can explore their surroundings (Nicol, 2015). Maternal care by a foster mother was shown to reduce fearfulness in an OF test in layer chicks (Rodenburg et al., 2009).

A mother hen provides more than a warm, dark place to rest; she also guides the chicks' feeding behaviour, among others (Nicol, 2015). However, fearful mother hens may also cause their chicks to be more fearful (Houdelier et al., 2011). Dark brooders may therefore be a good substitute, and are more feasible under commercial circumstances. The studies in laying hens show dark brooders can have a long-lasting effect on chickens' feather pecking behaviour, possibly because they are applied in early life, when the brain is still developing and the chick is still maturing (Nicol, 2015), conditions under which stimulation may have a long-lasting impact. Until now studies regarding the effect of dark brooders have only been performed with laying hens, and have not included persistence of the differences after the period with access to the brooders.

Dark brooders may have other effects on chicks' development and free-range use. For example, chicks with brooders become habituated to going from a dark to a light environment, which may mimic the transition between the house and the range later in life. They also have more freedom to choose between different environments, perhaps leading to more controllability. This is beneficial for their welfare because it reduces stress levels (Wiepkema and Koolhaas, 1993). In addition, they may learn that exploration is rewarding, because it allows them to discover the dark brooders for resting, and areas outside the dark brooders for activities such as feeding, drinking, foraging and interacting with conspecifics. These factors, together with reduced fearfulness, may all contribute to increased free-range use later in life.

The aims of this study were 1) to test how access to dark brooders early in life affected fearfulness, free-range use and behaviour later in life, 2) to test how overhangs adjacent to the pop holes affected free-range use and behaviour, and 3) to test whether presence of dark brooders and overhangs interacted with chickens' preference for shelter type (SRCW or AS). We hypothesised that dark brooders and overhangs would increase free-range use, that dark brooders would increase the number of birds outside and far from the house as a result of reduced fearfulness, and that the effects of brooders and overhangs would be largest in AS because fearfulness would play a bigger role in a less sheltered environment.

Material and Methods

Animals and housing

All animal procedures were approved by the Ethics Committee of Flanders Research Institute for Agriculture, Fisheries and Food (ILVO, Merelbeke, Belgium). In order to have replications of the treatments over time, three production rounds with 440 birds (Sasso XL451; the most common breed in organic broiler production in Belgium) per round were completed. Round 1 lasted from March 7 until May 16, round 2 from May 23 until August 1, and round 3 from August 8 until October 17 2016. No experiments were done in the winter because the mobile houses could not be heated, which could cause problems with the water supply and impair chicken welfare. During days 0 - 25 chicks were housed indoors in four pens of 3 x 4.4 m (110 birds per pen). The male:female ratio was 50:50 in each group. Each pen contained one bell drinker (circumference 100 cm) and one feeder (circumference 130 cm). Feed was provided in three phases: starter from week 1 - 3, grower from week 4 - 7, and finisher from week 8 – 10. The feed was produced by ILVO, see Chapter 5 for the exact composition). Two of the pens contained three 150 W infrared lamps; the other two were fitted with a dark brooder. The dark brooder was a wooden frame (l x w x h: 120 x 60 x 40 cm) with rubber fringes on the sides, and a wooden panel equipped with two 150 W ceramic heat panels (that only produced heat, no light) on top (Figure 4.1). Temperature inside the brooder was regulated by a thermostat (Thermo 2, Bio Green, Bischoffen-Oberweidbach, Germany) and was reduced gradually over time, from 29.2 ± 5.2 °C in week 1 to 25.8 ± 4.1 °C in week 4 under the brooders, and from 27.6 ± 2.6 °C in week 1 to 23.8 ± 3.3 °C in week 4 in the rest of the pen. The room temperature was controlled manually.

On day 25, the birds were moved in the same groups to four mobile houses on a 100 x 100 m field (Figure 4.2). To move them, the birds were caught manually and placed in transport crates (25-30 birds per crate), after which they were transported by tractor over a distance of 400 m, and released into the mobile houses. Outdoor access was provided from day 28 onwards. Pop holes were opened at 0800 h and closed around sunset (between 1844 h and 2200 h depending on the season). All groups had access to both grassland with AS and SRCW (Figure 4.2). SRCW was planted in double rows at high density; distance between two double rows was 1.5 m, distance between two single rows within a double row was 0.75 m, and distance between trees within a single row was 0.6 m. Average height of the SRCW in the year of the experiment was 5.8 m. The distance between the houses and the SRCW or AS was 1 – 2 m. Houses were 4.1 x 4.25 m, contained two bell drinkers and two feeders each (both will a circumference of 110 cm), with solid floors covered with wood shavings (Plospan, Waardenburg, Nederland). Two of the four houses had overhangs; these were wooden constructions (l x w x h: 100 x 100 x 50-100 cm; Figure 3). Two overhangs were placed per house, in front of the pop hole on either side of the house. The overhangs were switched between houses every week to increase the statistical power of the experiment: house I (dark-brooded birds) and house II (non-brooded birds) had overhangs in weeks 6, 8 and 10; house III (dark-brooded birds) and house IV (non-brooded birds) in weeks 5, 7 and 9. This switch was always done on Friday evening, so that the birds could acclimatise to the new situation before observations of free-range use started on the following Monday.

Behavioural tests

On day 18, 100 birds (25 per group) per round were given a neck tag with a unique number. The number of birds was chosen based on the number of birds that could be tested in this time frame. On day 22 and 23, these birds were subjected to an OF test. Prior to the test a small group of birds (10 – 15) were fenced off from the rest of the group, to facilitate catching of the birds in a calm manner. The birds were caught individually and transported by hand to the novel arena, which was just outside their home pen, in the same room. After catching five birds from the fenced-off group, the rest of the birds were released and a new group was placed in the fenced-off area. During the OF test the bird was placed in a novel arena (1.5 x 1.5 m) for 5 minutes. Their behaviour was video recorded and scored in Observer XT (Noldus, Wageningen, the Netherlands) using the ethogram in Table 4.1, from which ‘latency to first movement’ (walking or jumping), ‘latency to vocalise’, and ‘number of transitions between behaviours’ were derived as extra variables. Using Ethovision (Noldus, Wageningen, the Netherlands) the OF arena was virtually divided into 16 squares measuring 37.5 by 37.5 cm, and the following variables were analysed: total distance moved, latency to move from the initial square to another square, percentage of time spent in squares alongside the edge and in the centre of the arena, and number of transitions between squares.

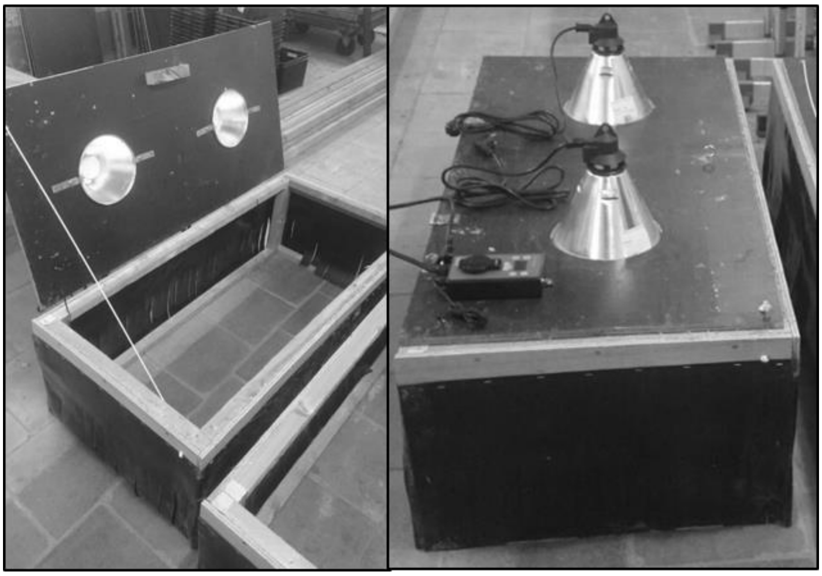


Figure 4.1 Dark brooder (l x w x h: 120 x 60 x 40 cm; left: opened, right: closed) with two ceramic heat lamps (150 W each) in the wooden panel on top, and rubber fringes around the sides to create a dark, secluded area.

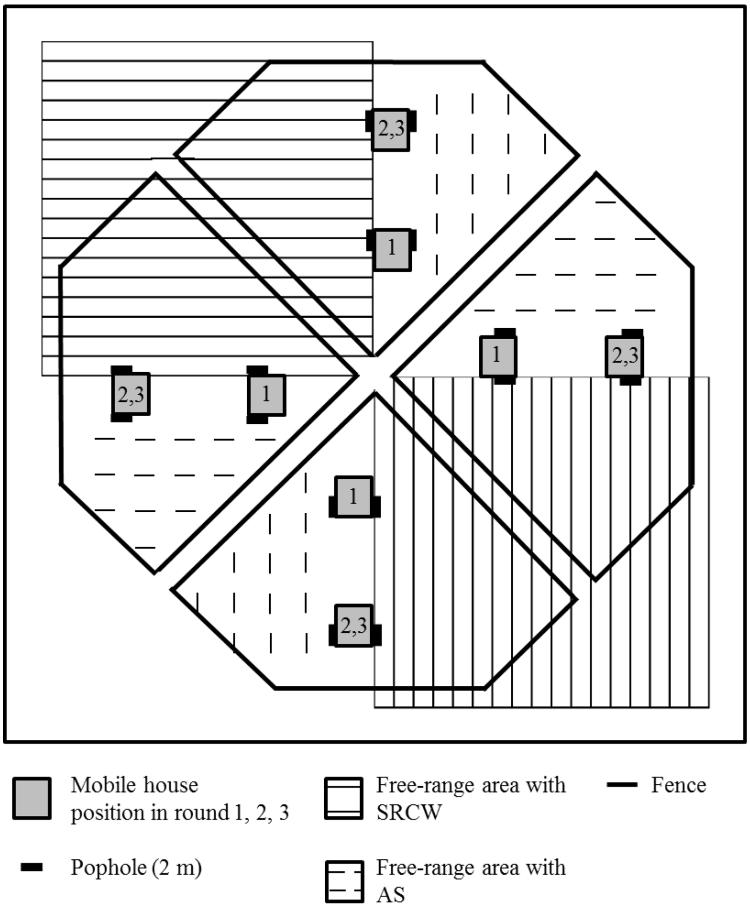


Figure 4.2 Schematic top view of the experimental field (outer edges 100 x 100 m) with four mobile stables, short rotation coppice willows (SRCW) and grassland with artificial shelter (AS).

On day 23 (birds that had been subjected to an OF test on day 22) and 24 (birds that had been subjected to an OF test on day 23) the same 100 chickens used in the open field test were subjected to a TI test. The same manner of catching as for the OF test was used, and the TI cradle was just outside the birds' home pen. The birds were placed on their back in a U-shaped cradle, were fixated for 15 seconds and then released (Jones, 1986). The number of inductions needed to successfully induce TI (bird remained on its back for at least 10 s) was recorded, with a maximum of three, and the TI duration was recorded (time to righting of the bird), with a maximum of 300 s.

Free-range use

Free-range use visual observations were performed every week day around 0900 h, 1300 h and 1700 h, by an observer standing approximately 10 m from the house after an acclimation period of 2 minutes. The number of birds outside was counted using scan sampling around 0900 h, 1300 h and 1700 h (exact time depended on the group of the bird, as the order in which the groups were observed was rotated), and for each bird observed outside the area, it was noted which area it was in (AS or SRCW), the distance from the house (0-2 m, 2-5 m or >5 m), and its behaviour was noted using scan sampling (Altmann, 1974). Observations were always conducted starting with the birds closest to the house in one of the shelter types, alternating between starting in AS or SRCW. Behaviours that were scored were walking, running, sitting or lying, standing, foraging, preening, dust bathing and aggressive behaviour. The same descriptions for the behaviours were used as during the OF test (Table 4.1). In addition, foraging was defined as scratching the ground with feet, pecking in the soil, or eating plant material. Dust bathing was defined as a bird sitting or lying down while fluffing dust through the plumage, accompanied by wing-shaking, head rubbing, bill-raking and scratching with one leg (van Liere, 1991). Aggression was defined as a negative interaction with other birds, which included aggressive pecks, leaps, chases, stand-offs, threats and fights (Estevez et al., 2002).

Data analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Data are presented as LS means ± 95% CI unless stated otherwise. Continuous variables were considered sufficiently normally distributed based on the graphical evaluation (histogram and QQ plot) of the residuals. Statistical significance was evaluated at $P < 0.05$, while $P < 0.1$ was considered a tendency. Factors with P values > 0.1 were removed from the final models (one at the time, starting with the factor with the highest P value), except for ‘dark brooders’ and ‘overhangs’ and factors that were part of significant interactions. In case of post-hoc pairwise comparisons, the Tukey-Kramer adjustment for multiple comparisons was used at a total significance level of 0.05. The results of the analyses are displayed with their corresponding F values and degrees of freedom (numerator and denominator).

Figure 4.3 Upper left and right: mobile house with open pop hole, with and without adjacent overhangs. Lower left: grassland with artificial shelters. Lower right: short rotation coppice.

Data from the behavioural tests were analysed using generalised linear mixed models with dark brooder (yes/no) as fixed effect and production round and pen within production round as random effects. In the OF test, scratching and wing flapping never occurred, and were therefore not included in the statistical analyses. In order to obtain normally distributed residuals, latencies to first activity, to first vocalisation and to leave their initial square during the OF test, and TI duration were all log-transformed. Of the OF test data, sitting, jumping, preening, pecking at the ground or wall occurred rarely so were dichotomised (yes = occurred, no = did not occur). The number of inductions for the TI test was also dichotomised (0 = one induction needed, 1 = more than one induction needed).

Table 4.1 Ethogram of behaviours scored during the open field test (performed on day 22 or 23, n = 100 chicks per round, 50 per dark brooder treatment group). States were expressed in duration (s), events in counts (number of times they occurred).

Behaviour	Description
States	
Stand	Stand upright on both feet
Sit or lie	Contact with floor with belly or side
Walk	Forward movement, at least one foot on the ground at anytime
Jump/fly	Upward and forward movement, feet do not touch the floor
Events	
Wing flap	Walking or standing while spreading and flapping the wings
Preen	Smoothing or cleaning feathers with the beak or bill
Defecate	Drop faeces
Vocalise	Make a sound
Scratch	Scratch body with one of the feet
Peck floor/wall	Peck the floor or wall of the arena with the beak

For the data on free-range use, the percentage of chickens observed outside per shelter type and distance from the house were grouped per week, and this average of all observations per week was used for statistical analysis. The data were analysed using a logistic mixed regression model with a first-order autoregressive covariance-structure to correct for multiple observations over time within the same house. Dark brooder (yes/no), overhangs (yes/no), shelter (AS/SRCW), distance from the house (<2 m, 2-5 m, >5 m) and week (5-10) and their interactions were included as fixed effects, as well as a three-way interaction between shelter, distance and week, and house within production round as random effect. Dark brooder and overhangs were never removed from the models as they were the primary focus of this study. After removal of other non-significant factors, the fixed factors that remained in the model were dark brooder, overhangs, shelter, distance from the house, week, week*shelter, week*distance and shelter*distance.

The behaviours of the outside chickens (percentage of the chickens observed outside that performed the behaviour) were also analysed using a generalised linear mixed model with dark brooder, overhangs, shelter, distance from the house, week and their interactions as fixed effects, and house within production round as random effect. Because ‘running’ occurred rarely, it was grouped with ‘walking’ to form a new variable ‘locomotion’. Again, non-significant factors were removed from the models, but dark brooder and overhangs were always retained.

Results

Behavioural tests

The presence of a dark brooder had no significant effect on the variables scored in the OF and TI tests (Table 4.2). Only the percentage of birds that jumped in the OF test tended to be higher in the groups without a dark brooder ($P = 0.075$).

Free-range use

The percentage of chickens outside was not influenced by presence of dark brooders in their early life (no brooder: 34.9% (95% CI: 27.7 – 42.1) vs. with brooder: 31.7% (95% CI: 24.4 – 38.9), $F_{1,10} = 1.62$; $P = 0.232$) or by the presence of overhangs during the period with free-range access (no overhangs: 32.5% (95% CI: 25.8 – 39.2) vs. with overhangs: 34.1 (95% CI: 27.4 – 40.8), $F_{1,7} = 0.52$; $P = 0.496$). An interaction existed between week, distance from the house and shelter ($F_{2,408} = 8.83$; $P < 0.001$; Figure 4.4). Birds preferred SRCW over AS at all ages and at all distances from the house except in week 5, which was the first week with outdoor access. The mean percentage of chickens outside increased with age of the birds in SRCW, but not in AS. In SRCW, the birds showed an increasing preference for areas farther from the house over time; this was not the case in AS.

Behaviour of birds outside

Behaviour of the chickens observed outside the house was influenced by several factors. The presence of dark brooders in their early life or overhangs during the period with free-range access were either not or rarely associated with behaviour, respectively (Table 4.3). Standing tended to be affected by an interaction between week of age and shelter type, but no significant pairwise differences were found. Standing occurred most at <2 m from the house ($P < 0.001$; Figure 4.5). Locomotion tended to occur more in the presence of overhangs, and more in SRCW than in AS (Figure 4.5). Sitting increased over time and occurred more in SRCW than in AS in all weeks except week 5 (all $P < 0.001$; Figure 4.5; for comprehensions only week 5 and 10 are shown). Sitting occurred more often in birds close to the house, although in SRCW no difference between <2 m and 2-5 m was observed (all other $P < 0.031$; Figure 4.5). Preening increased with age (+0.1% per week; Table 4.3). Foraging occurred more often in AS than in SRCW, and this difference increased with age (all $P < 0.011$; Figure 4.5). In AS, it occurred most at >2 m from the house, while in SRCW it was also higher at >5 m than at 2-5 m (Figure 4.5). Agonistic behaviours occurred more in SRCW than in AS (Figure 4.5).

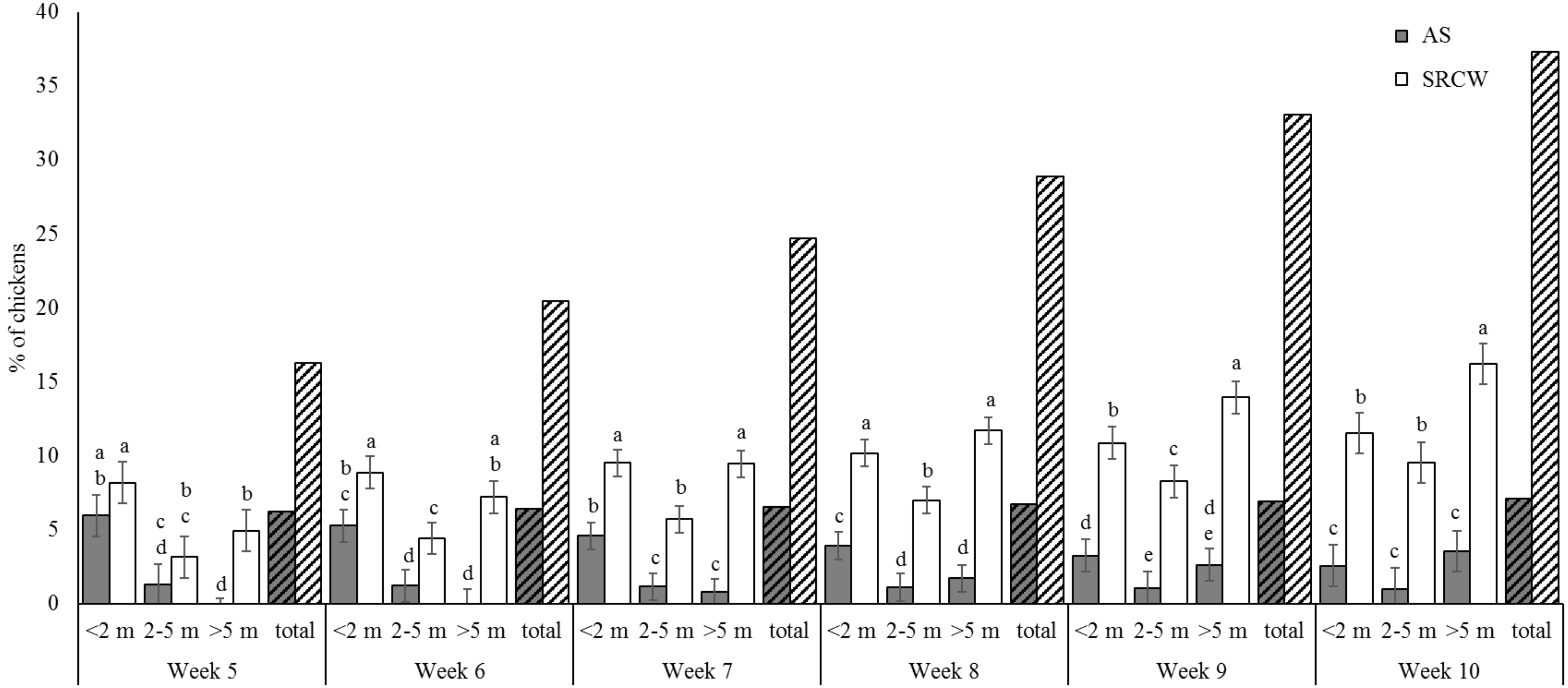


Figure 4.4 Percentage (LS means ± 95% confidence interval) of chickens outside per distance from the house, shelter type (AS = grassland with artificial shelter; SRCW = short rotation coppice willows) and week of age. Bars with different superscripts differ significantly within week of age. The striped bars represent the sum of the four previous bars (per distance from the house) for that shelter type and week of age.

Table 4.2 LS means and 95% confidence intervals (CI) of variables scored during the open field (on day 22 or 23) and tonic immobility tests (on day 23 or 24) for chicks reared with and without a dark brooder present (n = 100 chicks per round, 50 per treatment group).

	Brooder present		Brooder absent				
	Mean	95 % CI	Mean	95% CI	DF	F value	P value
Open field test							
Stand (s)	229	200 - 259	225	196 - 255	1,2	0.60	0.521
Walk (s)	68	39 - 97	72	43 - 100	1,2	0.74	0.479
Vocalise (number)	229	108 - 351	227	105 - 348	1,2	0.02	0.904
Defecate (number)	1.0	0.2 - 1.8	1.0	0.2 - 1.9	1,2	0.04	0.854
Sit (% of birds that showed this at least once)	2.6	0.8 - 8.6	2.0	0.5 - 7.5	1,10	0.12	0.731
Jump (% of birds that showed this at least once)	24.4	15.8 - 35.8	38.6	27.4 - 51.0	1,10	3.94	0.075
Preen (% of birds that showed this at least once)	14.6	8.4 - 24.2	18.3	11.1 - 28.8	1,10	0.49	0.501
Peck (% of birds that showed this at least once)	68.7	59.4 - 76.6	72.0	62.9 - 79.6	1,10	0.38	0.552
Transitions between behaviours[1] (number)	55	43 - 67	59	47 - 71	1,2	1.04	0.415
Distance moved (cm)	1757	928 - 2590	1867	1034 - 2700	1,2	0.73	0.483
Latency to first vocalisation (s)	7	3 - 13	6	3 - 13	1,2	0.03	0.879
Latency to first activity (s)	12	4 - 32	14	5 - 37	1,2	0.27	0.653
Latency to leave initial zone (s)	18	56 - 56	20	6 - 64	1,2	0.31	0.632
% of time spent in the outer zones	23.4	16.5 - 30.4	24.5	17.5 - 31.4	1,2	1.04	0.416
% of time spent in the centre zones	76.6	69.6 - 83.5	75.5	68.6 - 82.5	1,2	0.26	0.659
Transitions between zones (number)	49	21 - 77	53	26 - 81	1,2	1.50	0.345
TI test							
TI Duration	43	14 - 134	50	16 - 154	1,2	1.26	0.378
TI inductions (% of birds needing >1 induction)	37.3	24.6 - 52.2	33.1	21.1 - 47.7	1,10	0.24	0.636

[1] *Transitions between behaviours are defined as the number of transitions between different states (standing, sitting or lying, walking and jumping).*

Table 4.3 Overview of the effects of brooders (yes/no), overhangs (present/not present), week (5-10), shelter (artificial shelter/short rotation coppice), distance (<2 m/2-5 m/>5 m) and their interactions on outside chicken behaviours. DF = degrees of freedom (numerator, denominator), F = F-test statistic.

	Stand			Locomotion			Sit			Preen		
Factors	DF	F	P	DF	F	P	DF	F	P	DF	F	P
Brooder	1,10	0.62	0.451	1,10	0.04	0.850	1,10	0.61	0.452	1,10	2.07	0.181
Overhangs	1,7	0.39	0.552	1,7	4.20	0.080	1,7	0.05	0.836	1,7	0.67	0.439
Week	1,385	0.00	0.995	1,388	12.99	<0.001	1,383	114.67	<0.001	1,387	5.36	0.021
Shelter	1,11	4.18	0.066	1,11	4.29	0.063	1,11	12.28	0.005			
Distance	2,22	33.40	<0.001				2,22	54.76	<0.001	2,22	8.75	0.002
Week*shelter	1,385	4.44	0.036				1,383	38.57	<0.001			
Shelter*distance							2,21	5.17	0.015			

	Dust bathe			Forage			Aggression		
Factors	DF	F	P	DF	F	P	DF	F	P
Brooder	1,10	1.46	0.256	1,10	0.00	0.994	1,10	0.13	0.724
Overhangs	1,7	0.02	0.894	1,7	1.84	0.217	1,7	0.48	0.512
Week				1,383	60.68	<0.001			
Shelter				1,11	1.02	0.335	1,11	7.76	0.018
Distance				1,22	81.29	<0.001			
Week*shelter				1,383	11.90	<0.001			
Shelter*distance				1,21	5.15	0.015			

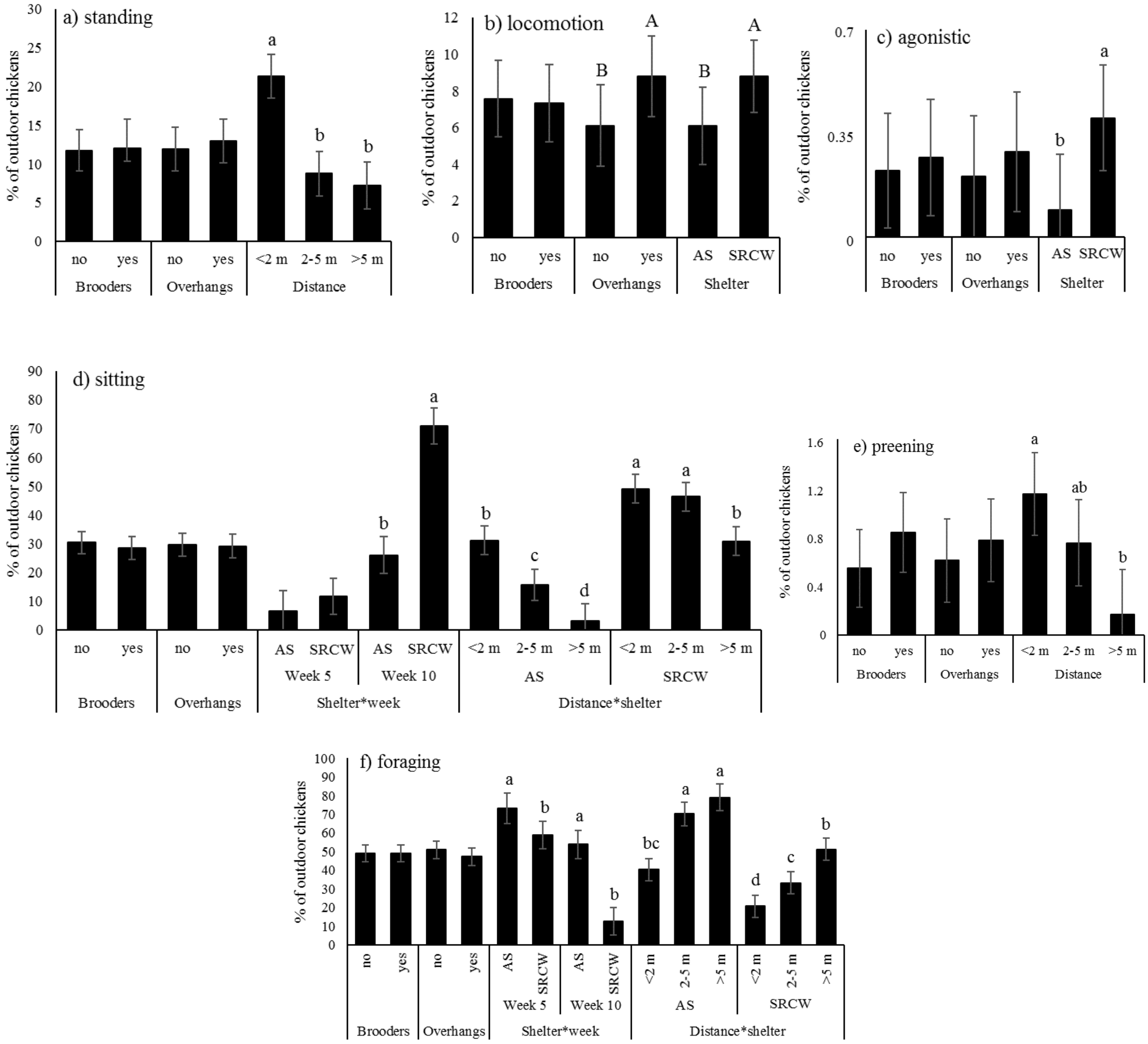


Figure 4.5 Behaviours expressed as the percentage (LS means SEM) of birds outside performing them, divided into groups with and without brooders and overhangs and where significant, week, shelter type and distance from the house and their interactions. Bars without common lower or upper case superscripts within a main effect or interaction indicate a significant difference ($P < 0.05$), or a tendency ($P < 0.1$), respectively, except for interactions including ‘week’ because ‘week’ was included in the model as a continuous variable, so no post-hoc pairwise comparisons could be made. AS = grassland with artificial shelter, SRCW = short rotation coppice willows.

Discussion

The presence of dark brooders during the first 4 weeks of life did not reduce chickens' fearfulness or explorative behaviours (walking, pecking at the arena walls or ground) as measured in the OF and TI tests. Only the percentage of birds jumping at least once in the OF test tended to be higher in the groups without dark brooders. Further, use of dark brooders did not affect the number of chickens outside or their distribution on the range during the free-range period. Also, the scored behaviours of outside birds were not affected by dark brooder rearing, including explorative behaviours such as walking and foraging, although differences in short duration behaviours such as aggression or behaviours that occur infrequently such as dust bathing may not have been recorded due to the instantaneous sampling method. This may be expected to be higher in brooded chickens because they learned the advantages of exploration, since the brooders provide them with different environments to discover.

The jumps in the OF test were often against the wall of the OF arena; they probably do not arise from a higher general activity level in non-brooder birds because other active behaviours such as walking did not differ between treatment groups. These jumps may have been escape attempts, which are less often observed under fear-eliciting conditions (Gallup and Suarez, 1980; Suarez and Gallup, 1983), so this may indicate higher levels of fearfulness in the dark-brooded birds. Alternatively, they may have been an indication of a higher motivation for social reinstatement (Forkman et al., 2007). Gilani et al. (2012) suggested that dark-brooded hens have a calmer nature; they found that hens reared with a brooder more often ignored humans during an approach test, although a novel object test gave no conclusive results. In a study by Riber and Guzman (2016), layer chicks were provided with dark brooders from day 0-41, and birds with brooders had shorter TI durations at week 4, 14 and 26 and were generally less often engaged in locomotion in the home pen. The brooders are thought to simulate certain aspects of a broody hen, which has been shown to reduce fearfulness in laying hens (Campo et al., 2014; Perré et al., 2002; Rodenburg et al., 2009; Shimmura et al., 2010). However, research on the effect of maternal care and dark brooders on broiler chickens is lacking. There are indications that broiler chickens are generally less fearful than laying hens (Keer-Keer et al., 1996; Saito et al., 2005), so a fear-attenuating treatment may affect them less, but these studies used fast-growing broilers which differ from the slower-growing hybrids such as the one used in this study.

Another difference between the results presented by Riber and Guzman (2016) and those of the present study was the temperature difference under the brooders compared to the ambient temperature in the room. In the study by Riber and Guzman (2016), the temperature under the brooder was considerably higher than the ambient temperature until day 28 (10-12 °C higher in the first 3 days, then lowered gradually), while in the present study, these temperatures were much more similar. In the current study the pens with and without brooders were located in the same room, so the ambient temperature had to stay within the thermal comfort zone for the chicks without brooders (Meltzer et al., 1982; Sasso, 2014). This may have caused the brooders to be less attractive as compared to the rest of the pen. It is possible that the chickens did not make enough use of the brooders, although informal observations indicated that they did use them frequently.

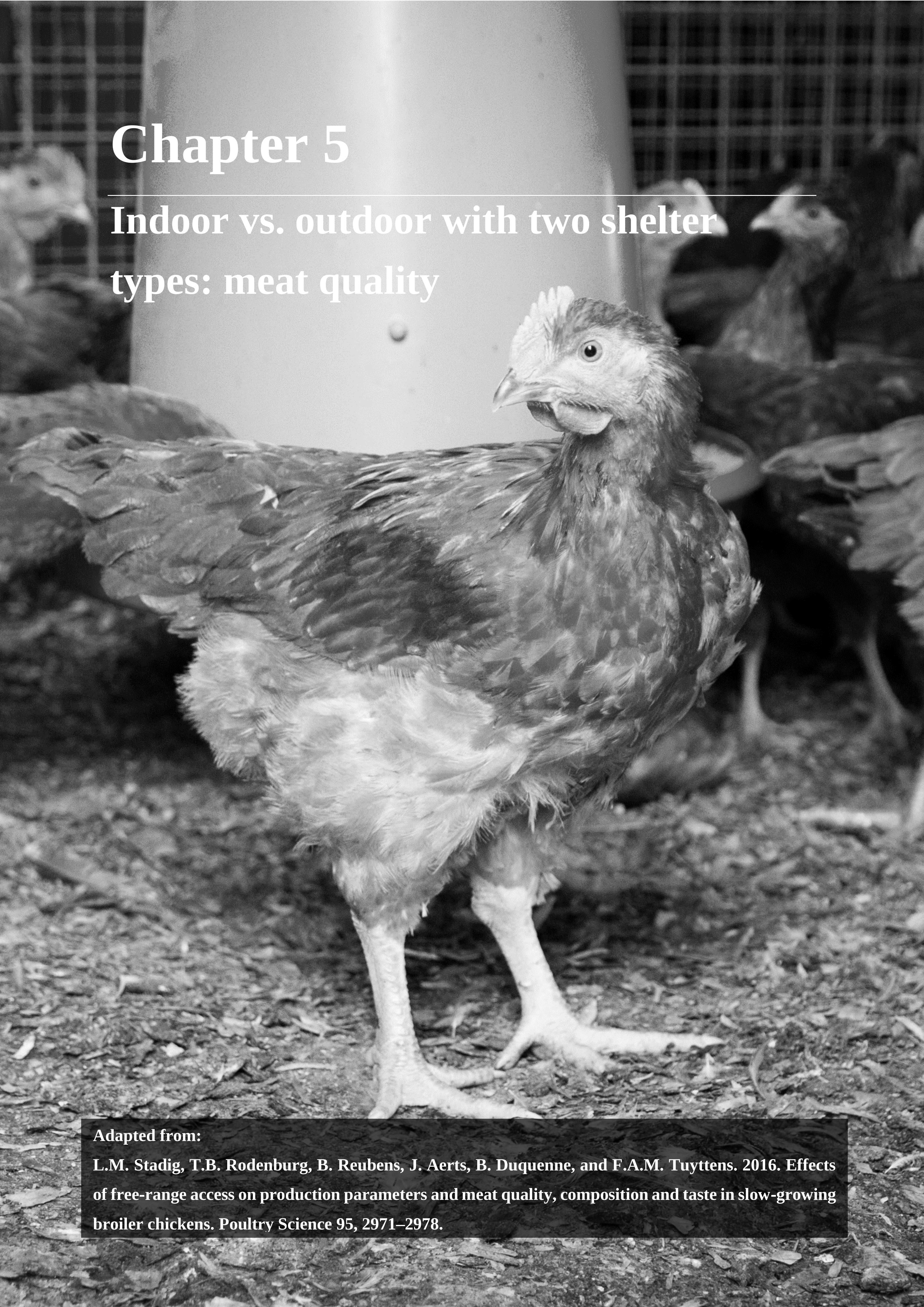
Overhangs did not affect the number of chickens located outside or the distribution of the flock on the range. Overall, free-range use in this study was high in both treatment groups, making it more difficult for either overhangs or dark brooders to have a positive effect. One explanation for this may have been that the transition between the house and range may already have been gradual enough in the houses without the overhangs, with small differences between light intensity inside and outside. The pop holes already had a small overhang in front of them (i.e. the hinged door of the pop hole). Also, the mobile houses were quite small, so the probability of a bird coming in proximity of a pop hole was high. Small flocks of laying hens have been shown to range more than large ones (Bestman and Wagenaar, 2003; Hegelund et al., 2005; Whay et al., 2007). The exact reasons for this remain unclear, although it has been suggested to be due to having to cross a smaller distance to reach the range (Chielo et al., 2016). Alternatively, the lack of effect of the overhangs could be due to the fact they were provided only every other week, which was done to increase the statistical power of the experiment. If they would have been provided during the entire period with free-range access, this may have yielded different results. This should be tested in future research. Future studies could also be aimed at clarifying the relationship between group size and free-range use, e.g. by testing differences between light intensity inside and outside.

Locomotion tended to occur more when overhangs were present. It is possible that the overhangs facilitated more traffic between the house and the range, but without resulting in more birds staying outside. However, it may then be expected that this effect is mainly seen in the areas closest to the house, but no interaction between presence of overhangs and distance from the house was found. The higher occurrence of aggression could be due to a higher number, thus a higher density of birds in this area, increasing the chance that a social conflict will occur, due to more encounters with other birds. Alternatively or related to that, more competition for highly valued resources such as insects or worms could occur in SRCW.

It was clear that chickens had a preference for SRCW over AS, in agreement with previous studies (Chapters 2 and 3 of this thesis). The observation that free-range use increased with age, especially in areas farther from the house in SRCW, also corresponds with earlier findings (Chapter 3 of this thesis). This can be attributed to either decreasing fearfulness and more habituation to the range over time or perhaps to a depletion of vegetation or other resources close to the house. Nevertheless, the finding that a higher percentage of foraging was observed in AS, but the birds did not range farther over time in this shelter type, seems to contradict the depletion-option. However, it is important to note that all behavioural data are expressed as a percentage of birds in that particular area (shelter type and distance from the house), and that the absolute number of birds in AS was always lower than in SRCW. This means that even if a higher proportion of the chickens in AS were foraging, a higher absolute number of chickens will have foraged in SRCW, perhaps leading to faster depletion of the vegetation. In addition, there was less ground cover in SRCW due to the dense foliage of the willows blocking the sunlight, so depletion could have occurred faster. Nevertheless, foraging motivation was probably not the only motivation to range far from the house in SRCW, as a higher percentage and number of birds was also observed to be sitting farther from the house in SRCW than in AS. This corresponds with findings of Stadig et al. (Chapter 3 of this thesis). These findings may be because the birds felt safer in SRCW, or the SRCW had a more comfortable microclimate such as less solar radiation (as slow-growing broiler chickens avoid direct sunlight; Chapter 3 of this thesis).

Conclusions

The present study found no clear effect of dark brooders on slow-growing broiler chickens' fearfulness, free-range use and behaviour, and little effect of overhangs on free-range use and behaviour. This was possibly due to less fearfulness in broiler chickens as compared to laying hens, where other studies showed fear-attenuating effects of dark brooders. Alternatively, the relatively high free-range use across all four treatment groups (perhaps due to small mobile houses and suitable shelter) may have made it more difficult to show effects of additional measures to improve range use. The chickens showed a clear preference for dense vegetation over artificial shelter, and ranged farther from the house in SRCW. Therefore, it can be recommended that in terms of free-range use, such dense vegetation is to be used more in free-range chicken production, although further research is needed to clarify which aspects of the vegetation (e.g. density or canopy cover) are key in attracting chickens to the range.



Chapter 5

Indoor vs. outdoor with two shelter types: meat quality

Adapted from:

L.M. Stadig, T.B. Rodenburg, B. Reubens, J. Aerts, B. Duquenne, and F.A.M. Tuyttens. 2016. Effects of free-range access on production parameters and meat quality, composition and taste in slow-growing broiler chickens. Poultry Science 95, 2971–2978.

Abstract

Demand for meat from free-range broiler chickens is increasing in several countries. Consumers are motivated by better animal welfare and other product attributes such as quality and taste. However, scientific literature is not unanimous about whether free-range access influences quality, composition and taste of the meat. Because chickens normally do not use free-range areas optimally, it is possible that provision of more suitable shelter will lead to more pronounced differences between chickens raised indoors and outdoors. In this study, an experiment with two production rounds of 600 slow-growing broilers each was performed. In each round, 200 chickens were raised IN, 200 had free-range access to grassland with AS, and 200 had free-range access to SRCW. Free-range use, FI and growth were monitored, and after slaughter (d72) meat quality, composition and taste were assessed.

Free-range use was higher in SRCW than in AS chickens (42.8 vs. 35.1%, $P < 0.001$). IN chickens were heavier at d70 than AS and SRCW chickens (2.79 vs. 2.66 and 2.68 kg, $P = 0.005$). However, FI and FCR did not differ. Breast meat of chickens with free-range access was darker ($P = 0.021$) and yellower ($P = 0.001$) than that of IN chickens. Ultimate pH was lower (5.73 vs. 5.79; $P = 0.006$) and drip loss higher (1.29 vs. 1.09%; $P = 0.05$) in IN versus AS chickens. The percentage of polyunsaturated fatty acids (**PUFA**) was higher in AS than in IN meat (35.84 vs. 34.59%; $P = 0.021$). The taste panel judged breast meat of SRCW chickens to be more tender ($P = 0.003$) and less fibrous ($P = 0.013$) compared to that of AS and IN chickens, and juicier compared to the IN chickens ($P = 0.017$). Overall, free-range access negatively affected slaughter weight, but positively affected meat quality, taste and composition. Only few differences between AS and SRCW were found, possibly due to limited differences in free-range use.

Introduction

Demand for meat from free-range broiler chickens is increasing in several countries (Ministerie EL&I, 2012; Verbeke, 2012). Consumers believe that free-range access is important for broiler chicken welfare (de Jonge and van Trijp, 2013; Vanhonacker et al., 2016). Concerns about animal welfare is one driver for consumers to buy particular products (Davidson, 2003; Fearne and Lavelle, 1996; Hill and Lynchehaun, 2002; Vanhonacker et al., 2010). But welfare is not the only driver: a Belgian study showed that when buying chicken meat, product attributes such as healthiness, quality and taste are judged to be pivotal (Vanhonacker and Verbeke, 2009). Some consumers see animal welfare as an indicator for some of these other product attributes

(Harper and Henson, 2001). These attributes are often perceived to be superior in organic compared to conventional animal products (Hughner et al., 2007) for several reasons. In broiler chickens, organic production systems provide free-range access, but also use slower-growing hybrids. This choice of breed alone can influence meat quality parameters such as tenderness, regardless of the production system used (Ponte et al., 2008c).

Free-range access can possibly influence quality, composition and taste of broiler chicken meat, because the animals are likely to exercise more (Castellini et al., 2002b) and they have access to fresh and diverse plant and animal food sources. Several studies have investigated the effect of free-range access on meat quality, composition and taste, but results are not consistent. The only mostly consistent finding is that colour is found to be more yellow in meat from chickens with free-range access, possibly due to plant or carotenoid intake (Castellini et al., 2002b; Fanatico et al., 2007; Sales, 2014). Other parameters such as tenderness, WHC and protein content often yield conflicting results, with some studies reporting these aspects to be superior in free-range chicken meat (Jiang et al., 2011; Sun et al., 2013), while others report it to be inferior (Castellini et al., 2002b; Mikulski et al., 2011) or not significantly different (Chen et al., 2013; Wang et al., 2009).

In addition to the possible influences on meat quality, free-range access can also influence production parameters such as BW, FI and FCR. A possible reason is that as free-range chickens get more exercise, they will have a lower BW at slaughter and a higher FCR, which is confirmed by several studies (Castellini et al., 2002b; Wang et al., 2009). However, others found no differences (Fanatico et al., 2005b; Tong et al., 2014) or even better productive performance in the free-range groups (Ponte et al., 2008b, 2008c).

The varying level of free-range use may have contributed to these inconsistent findings. Free-range use by broiler chickens is often quite limited, with on average only 5 - 11% of the animals being outside at any given moment (Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). If free-range use were to increase, the potential effects on performance and meat characteristics might be more pronounced. Therefore, in the present experimental setup, we attempted to increase free-range use by providing different types of shelter. Shelter has been shown to be an important determinant of free-range use (Dawkins et al., 2003; Dal Bosco et al., 2014). However, it is not yet known what kind of shelter chickens prefer. Shelters can be divided into artificial and natural shelter, which could both have advantages, but since domestic chickens descend from jungle fowl, it is likely that they will prefer an environment with dense vegetation. The aim of this study was to investigate whether differences in production performance, quality, composition or taste of breast meat could be observed from chickens either kept indoors or given access either to grassland with AS or to SRCW (to stimulate free-range use).

Materials and Methods

Animals and housing

In 2014, an experiment with two 10-week production rounds was performed (May-July, July-Oct), each with 600 slow-growing mixed-sex broiler chickens. The Sasso XL451 hybrid was chosen because it is the most common hybrid used in organic broiler production in Belgium, a country where organic broilers are nearly the only ones with free-range access. Feed was provided in three phases: starter from week 1 - 3, grower from week 4 - 7, and finisher from week 8 – 10 (the feed was produced by ILVO; Table 5.1). Both feed and water were available *ad libitum*. All experiments were performed at the Institute of Agricultural and Fisheries Research (ILVO), which is not a certified organic facility. All experimental procedures were according to Belgian legislation and approved by the Animal Ethics Committee of the ILVO.

Phase 1 All chickens were housed indoors from day 0 to day 28 (round 1) or 21 (round 2) in four groups of 150 animals per round (25 birds / m^2). The light regime was 24L:0D for the first five days, and 16L:8D for the remaining days. Litter consisted of 10 cm of wood shavings.

Phase 2 Per round, 400 randomly selected birds (200 males, 200 females) were moved to mobile chicken houses, and were given free-range access from day 39 (round 1) or day 28 (round 2) until day 72. In round 1, the day of moving to the mobile houses and start of free-range access were delayed due to construction problems with the flooring of the mobile houses. The four mobile houses were located on a 100 x 100 m plot (Figure 5.1). The remaining 200 chickens were housed indoors in four groups of 50 birds until day 72 (IN). The light regime was maintained similar to that of daylight. All housing systems had the same litter (10 cm of wood shavings).

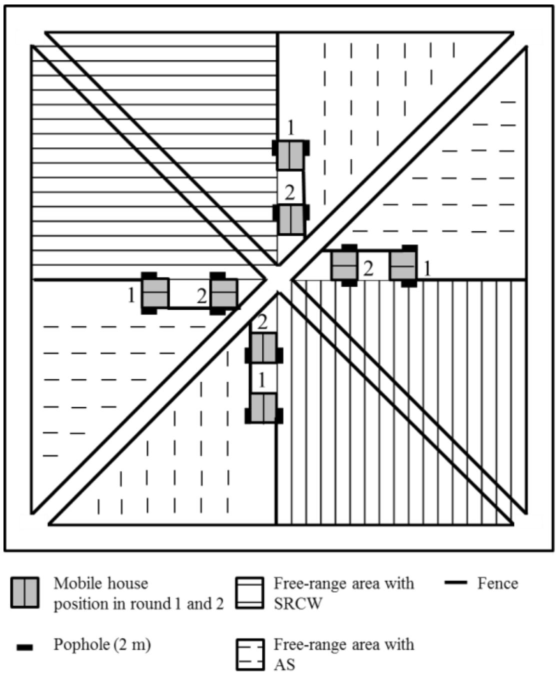


Figure 5.1 Top view of the experimental site (100 x 100m) with short rotation coppice willows (SRCW) and grassland with artificial shelter (AS).

Each of the four mobile houses was divided into two halves, with each half housing 50 birds. Per mobile house, one of the two groups of chickens was provided with free-range access to grassland with AS (21 wooden A-frames, l x w x h: 2.5 x 1.25 x 1.5 m; Figure 5.2). A grass (mixture of *Lolium perenne* (50%), *Poa pratensis* (20%), *Festuca rubra* (15%) *and Phleum pratense* subsp. *Pretense* (15%)) and clover (*Trifolium repens*) mixture was used on this field. The other group had access to SRCW with Swedish willow (*Salix* spp.) clones (Tora (*S. schwerinii*), Klara ((*S. burjatica* x *S. viminalis*) x *S. burjatica*) and Tordis (*S. schwerinii* x *S. viminalis*)), planted in spring 2013 at high density (15,000 trees/ha), in accordance with common practice for growing these trees for biomass production (75 cm between single rows, 150 cm between double rows, 60 cm between trees in each row; Figure 5.2). All free-range areas were completely vegetated. Each group had one pop hole of 2 x 0.5 m (L x H), which was opened in the morning between 0700 h and 0900 h and closed at sunset. All chickens, regardless of housing type (IN, AS or SRCW), had the same amount of indoor space available (12.5 birds / m^2). The mobile houses were repositioned between the production rounds to enhance use of a larger area of the field and minimise point pollution. Two production rounds were performed to increase statistical power, and to not limit the results to one specific season.

Table 5.1 Ingredients (%) and calculated dietary composition (% of dry matter) and fatty acid profile (% of dry matter) of the starter, grower and finisher feed

Ingredients	Starter	Grower	Finisher
Wheat	36.87	32.10	40.81
Soybean meal (crude protein 48%)	21.8	15.00	15.00
Corn	15.00	20.00	16.54
Rapeseed (crude protein 38%)	12.00	9.68	12.00
Soybeans	5.86	12.75	8.93
Animal fat	2.50	1.97	3.00
Soybean oil	1.53	1.00	1.00
Dicalcium phosphate	1.47	1.12	1.00
Sunflower (crude protein 32%)	1.30	4.74	0.00
Premix[1]	1.00	1.00	1.00
Limestone	0.35	0.30	0.39
NaCl	0.25	0.25	0.25
Na bicarbonate	0.09	0.09	0.09
Composition			
Metabolizable energy (MJ/kg)	11.25	11.30	11.70
Crude protein	24.92	24.38	22.83
Crude fat	7.99	8.55	8.68
Crude fibre	5.20	5.53	4.63
Lysin	1.31	1.26	1.18
Methionin + cysteine	0.88	0.85	0.82
Threonin	0.99	0.96	0.90
Ca	1.05	0.91	0.91
Available P	0.46	0.40	0.37
Na	0.15	0.15	0.15
K	1.12	1.10	1.01
Cl	0.23	0.24	0.23
Fatty acid profile			
C14:0	0.05	0.05	0.06
C16:0	1.23	1.18	1.38
C16:1	0.10	0.09	0.12
C18:0	0.48	0.45	0.55
C18:1	2.25	2.20	2.51
C18:2	2.68	3.12	2.78
C18:3	0.33	0.38	0.33
C20:5	0.01	0.00	0.01
C22:6	0.00	0.00	0.00

[1] *Per kg: vitamin A 1,349,997 IU, vitamin D3 299,999 IU, choline 60,000 mg, vitamin E 5,488 mg, nicotinic acid (B3) 3,000 mg, vitamin B3 (niacin) 1,500 mg, vitamin B2 500 mg, vitamin B6 400 mg, vitamin K3 250 mg, B1 200 mg, folic acid 100 mg, biotin 20 mg, B12 2 mg, Mn 9,590 mg, Zn II oxide 6,000 mg, Fe sulphate 4,920 mg, cupric sulphate 2000 mg, calcium iodate 120 mg, sodium selenite 36 mg.*

Figure 5.2 Left: free-range area with wooden A-frames. Right: free-range area with short rotation coppice willows.

Data collection

Free-range use Cameras recorded the number of chickens inside the mobile houses (Bushnell Trophy Cam, Bushnell, Kansas City, MO). One photograph per hour was taken and used to calculate the percentage of animals outdoors at that moment. In addition, live observations were performed. At 10 days in round 1 (day 43, 45, 50, 51, 52, 56, 57, 59, 63, 65), and 13 days in round 2 (day 29, 31, 36, 37, 38, 42, 43, 44, 49, 50, 51, 57, 58), the number of chickens using the free-range area was recorded once per group (always at 0900 h, 1300 h and 1700 h), and a distinction was made between those within 5 m from the house and those further away.

Growth and feed conversion Feed was provided in three phases: starter from week 1 - 3, grower from week 4 - 7, and finisher from week 8 - 10. FI was recorded per group, starting on the day that the birds were moved to the mobile houses. All groups were also weighed on that day and again on day 70.

Meat quality On day 72, chickens were transported for 45 min then commercially slaughtered using electrical water bath stunning. Carcasses were immediately chilled after processing. Forty-eight chickens per round (16 from each treatment group) were used to analyse meat quality. Per pen, two male and two female birds were selected based on their weight, which was not allowed to differ more than 0.2 kg from the average of all birds (measured during the welfare assessment in week 10, unpublished data). Average weights of the males and females were 2.83 ± 0.15 kg and 2.26 ± 0.15 kg, respectively.

Approximately 5 h after slaughter, the right breast fillet was removed from the carcass and weighed. Colour was measured in duplicate per fillet using a Miniscan EZ colorimeter (Hunterlab, Reston, VA) to record L* (lightness), a* (redness), and b*(yellowness) values. Temperature and pH were measured 24h later (pH-ultimate), in duplicate per fillet using a Portamess® 910 (Knick, Berlin, Germany). Fillets were then put in a plastic bag, hung for 24 h at 8 - 10 °C then blotted dry and weighed again to measure drip loss. Subsequently, fillets were vacuum packed and stored at -20 °C for 4 days. They were then defrosted for 24 h at 5 °C, blotted dry, weighed, and cooked in a warm water bath (80 °C) for 30 min. Afterwards they were blotted dry and weighed to record cooking loss. They were then stored at 5 °C for 24 hours, after which two cylindrical pieces of meat (parallel to the fibres, diameter 12 mm, at least 3 cm long) were cut from each fillet. These were used for a shear force test, using the Basic Force Gauge BFG 1000N (Mecmesin, Slinfold, UK). The maximum force needed to cut the piece of meat was recorded.

Meat composition The carcasses were kept at 8 - 10 °C until 24 h after slaughter. Then the left breast fillets were removed, vacuum packed, and stored at -20 °C. These were used for analysis. Crude fat was determined using ISO 6492 (1999). The N content of the carcass samples was determined according to the improved Kjeldahl method (ISO 5983-2, 2005). Water content was analysed using the ISO 1442 (1973) method. Ash content was calculated as 100 – moisture – crude protein – crude fat. A fatty acid profile ($C_{6:0}$ to $C_{24:1}$) was determined using gas chromatography coupled to flame ionization detection. After extraction and methylation, fatty acid methyl esters were identified and quantified using C*19:0* as an internal standard (according to Sukhija and Palmquist, 1988).

Sensory characteristics Per round, 78 chickens were selected for sensory analysis (ISO8589, 2007) on their breast fillets (26 per treatment group). These carcasses were kept at 8 - 10 °C until 24 h after slaughter. Then the breast fillets were removed, vacuum packed, and stored at -20 °C. Breast fillets were thawed at 4 °C for 36 h before being prepared for the sensory analysis. The meat was prepared in a convection oven (200 °C) until it reached a core temperature of 70 °C. It was left unseasoned so that only the taste of the meat would be judged. Each fillet was sliced into three portions of 40 - 50 g, excluding the outer (thinner) edges. Samples from the three treatments were immediately and simultaneously presented to trained and experienced panellists (the order of treatments on the plates differed among the panellists). A panel of 29 (round 1) or 25 (round 2) assessors were asked to judge the breast fillets according to seven characteristics, scored on a scale of 1 - 10: taste, aroma, tenderness, fibrousness, colour, juiciness, and appearance (*e.g.* for appearance: 1 = not attractive at all, 10 = very attractive). Samples were analysed blind: the panel members were unaware of the experimental treatments.

Data analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC). Data are presented as LS means ± standard errors, unless stated otherwise. Variables were analysed using a linear mixed model with treatment group and sex as fixed effects. For meat quality, fillet weight was added as fixed effect because the initial weight could influence variables such as drip or cook loss due to a different surface to content ratio. Production round and house within production round were included as random effects. For the sensory data, the analyses also corrected for repeated measures by including the ID of the panellist in the random part of the model. Variables were considered sufficiently normally distributed based on the graphical evaluation (histogram and QQ plot) of the residuals. Statistical significance was evaluated at $\alpha = 0.05$. Non-significant factors were removed from the models. In case of pairwise comparisons, the Tukey-Kramer adjustment for multiple comparisons was used at a total significance level of 0.05.

Results

Free-range use

Mean free-range use was higher in the SRCW than in the AS groups (42.8 vs. 35.1% of the chickens being outside at a given time; $F_7 = 233.7$, $P < 0.001$), and chickens from the former groups also ranged further from their house (10.6 vs. 4.1% of the outside chickens being further than 5 m from their house; $F_7 = 24.0$, $P = 0.002$). It was also observed that free-range use increased with age ($P < 0.001$). For more details on free-range use, see Chapter 1 of this thesis.

Production parameters

IN chickens were heavier on day 70 than AS and SRCW chickens ($P = 0.005$; Table 5.2). Mean FI per animal (during the mobile house period) ($P = 0.373$) and mean FCR ($P = 0.192$) did not differ between the treatments.

Table 5.2 Slaughter weight, feed per animal during their stay in the mobile house, and feed conversion per group of chickens either raised indoors (IN) or with free-range access: either grassland with artificial shelter (AS) or short rotation coppice willows (SRCW).

	Weight at day 70 (kg)	Feed per animal (kg)	Feed conversion (kg / kg)
IN	2.79 ± 0.02^{a}	6.08 ± 0.20	2.75 ± 0.11
AS	2.66 ± 0.03^{b}	5.91 ± 0.15	2.85 ± 0.06
SRCW	2.68 ± 0.03^{b}	6.14 ± 0.14	2.91 ± 0.08

$^{a\text{-}b}$ *Variables within a column lacking a common superscript differ significantly (P < 0.05).*

Meat quality

Colour of the breast meat differed between chickens with and without free-range access (Table 5.3). Breast fillets of chickens with free-range access were darker (P = 0.021) and yellower (P = 0.001) than those of IN chickens. Ultimate pH was lower for IN than for AS chickens (P = 0.006). Drip loss was higher in breast meat of IN chickens than in that of AS chickens (P = 0.05). No significant differences in redness, cooking loss and shear force were found among the three treatments.

Meat composition

There were no differences in fat, protein, moisture or ash content among the treatments (Table 5.4). The fatty acid profile differed between the treatment groups (Table 5.4). In general, birds with free-range access had higher levels of unsaturated fatty acids, which is a desirable characteristic for human health. $C_{15:0}$ was higher in the AS and SRCW groups compared to the IN group (P = 0.001 and P = 0.009), $C_{17:0}$ was higher in the AS than in the IN group (P = 0.026), and also tended to be higher in the SRCW group (P = 0.066), $C_{18:2n6}$ tended to be higher in the AS and SRCW than in the IN groups (P = 0.070 and P = 0.051), $C_{18:3n3}$ was higher in the SRCW than in the IN group (P = 0.012), and tended to be higher in the AS group (P = 0.056), $C_{22:6n3}$ tended to be higher in the AS than in the IN group (P = 0.055), and total PUFA were higher in the AS than in the IN group (P = 0.021). Total n3 and n6 were higher in AS than in IN (P = 0.036 and P = 0.020), as was the ratio between PUFA and monounsaturated fatty acids (**MUFA**; P = 0.039).

Sensory characteristics

Breast meat of IN, AS and SRCW chickens showed several differences, as scored by the taste panel on a scale of 0 - 10 (Figure 5.3). Regarding fibrousness, SRCW chickens scored lower, meaning that their meat was less fibrous than that of AS and IN chickens (4.3 ± 0.3 vs. 5.3 ± 0.4 and 5.4 ± 0.4; P = 0.013). Fillets of SRCW chickens were also scored to be juicier than those of IN chickens (6.3 ± 0.4 vs. 5.1 ± 0.4; P = 0.017). Furthermore, meat from SRCW chickens was scored as more tender than AS and IN chickens (6.7 ± 0.3 vs. 5.6 ± 0.4 and 5.4 ± 0.4; P = 0.003). The panel found no differences in taste, colour, appearance and aroma of the meat from the three treatments.

Table 5.3 Colour, pH, drip loss, cook loss and shear force of breast fillets of chickens either raised indoors (IN) or with free-range access: either grassland with artificial shelter (AS) or short rotation coppice willows (SRCW).

	Colour			pH-ultimate	Drip loss (%)	Cook loss (%)	Shear force (N)
	L*	a*	b*				
IN	55.3 ± 0.38^{a}	5.7 ± 0.21	13.4 ± 0.31^{b}	5.73 ± 0.02^{b}	1.29^{a}	16.6	22.5 ± 3.07
AS	54.0 ± 0.38^{b}	6.1 ± 0.21	14.9 ± 0.31^{a}	5.79 ± 0.02^{a}	1.09^{b}	16.7	22.7 ± 3.07
SRCW	53.9 ± 0.38^{b}	6.3 ± 0.21	14.7 ± 0.31^{a}	5.76 ± 0.02^{ab}	1.21^{ab}	16.6	23.1 ± 3.07

$^{a\text{-}b}$ *Variables within the same column without a common superscript, differ significantly (P<0.05).*

Table 5.4 Fat, protein, moisture, ash and fatty acid composition (%) of breast meat from chickens either raised indoors (IN) or with free-range access: either grassland with artificial shelter (AS) or short rotation coppice willows (SRCW).

		IN	AS	SRCW	P value
Crude fat		0.66	0.63	0.71	0.167
Crude protein		25.8	26.1	26.0	0.555
Moisture		73.2	73.1	72.9	0.425
Ash		0.33	0.28	0.27	0.953
C14:0		0.55	0.54	0.55	0.650
	C14:1n5	0.08	0.08	0.08	0.724
C15:0		0.08^{b}	0.09^{a}	0.09^{a}	<0.001
C16:0		24.68	24.39	24.52	0.302
	C16:1n7	2.16	1.92	1.95	0.171
C17:0		$0.19^{b;B}$	$0.22^{a;A}$	$0.21^{ab;A}$	0.021
C18:0		9.11	9.04	9.07	0.935
	C18:1n9	28.17	27.29	27.58	0.149
	C18:2n6	23.29^{B}	23.96^{A}	24.00^{A}	0.033
	C18:3n6	0.12	0.12	0.13	0.681
	C18:3n3	$1.41^{b;B}$	$1.52^{ab;A}$	$1.55^{a;A}$	0.011
C20:0		0.11	0.11	0.11	0.495
	C20:1n9	0.31	0.32	0.31	0.662
	C20:2n6	0.55	0.58	0.57	0.432
	C20:3n6	0.69	0.70	0.70	0.900
	C20:4n6	6.49	6.94	6.56	0.302
	C20:5n3	0.38	0.37	0.37	0.959
C21:0		0.22	0.20	0.21	0.414
C22:6n3		1.45^{B}	1.64^{A}	1.49^{AB}	0.051
Total					
	C6:0 - C15:1	0.68	0.67	0.68	0.762
	C16:0-C24:1	99.32	99.33	99.32	0.762
	n3	3.24^{b}	3.48^{a}	3.41^{ab}	0.097
	n6	31.13^{b}	32.30^{a}	31.95^{ab}	0.025
	SFA[1]	34.94	34.59	34.81	0.347
	MUFA[2]	30.46	29.57	29.88	0.246
	PUFA[3]	34.59^{b}	35.84^{a}	35.35^{ab}	0.028
Ratio					
	n6:n3	9.76	9.43	9.46	0.450
	PUFA:MUFA	1.13^{b}	1.22^{a}	1.19^{ab}	0.046

$^{a\text{-}b}$ Variables within a row lacking a common superscript differ significantly ($P < 0.05$) or, when in capital letters, tend to differ ($P < 0.1$).

[1] SFA: saturated fatty acids; [2] MUFA: monounsaturated fatty acids; [3] PUFA: polyunsaturated fatty acids

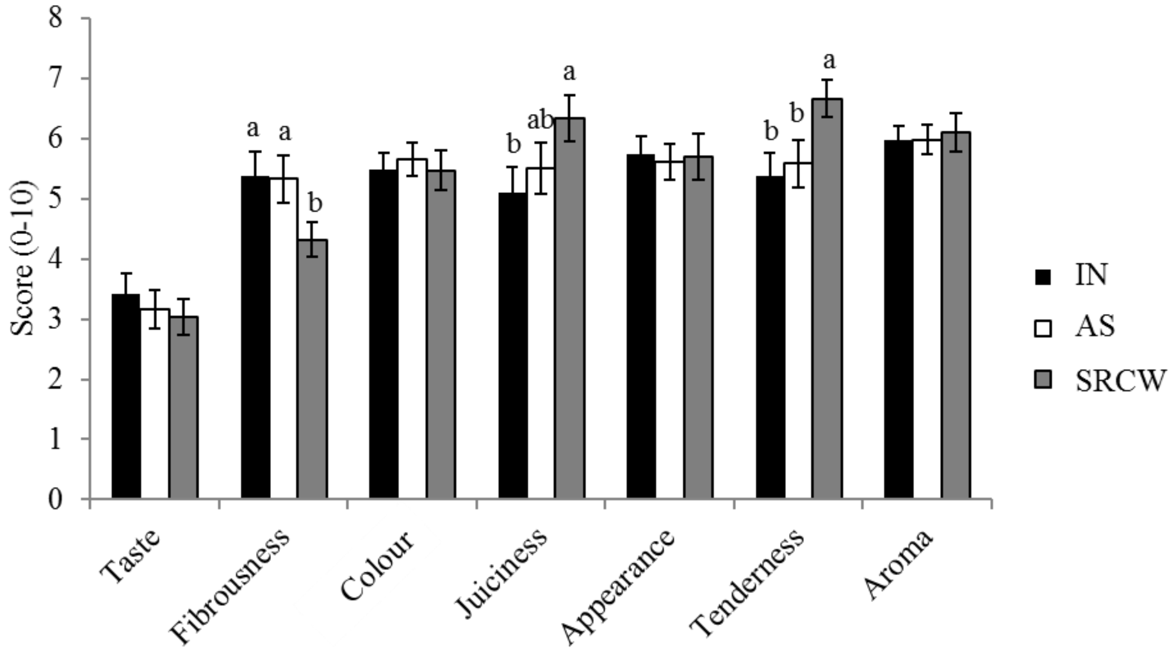


Figure 5.3 Sensory characteristics of breast meat from chickens raised indoors (IN) or with free-range access: either grassland with artificial shelter (AS) or short rotation coppice willows (SRCW). Characteristics were judged by a panel (n = 29 for round 1, n = 25 for round 2) on a scale from 0 to 10. Lack of a common superscript above the bars indicates a significant difference ($P < 0.05$).

Discussion

The present study aimed to increase free-range use by providing shelter. This was successful, as on average 35 - 43% of the chickens were found outside, with the highest free-range use in the SRCW group, which indicates that this shelter type is more suitable than the A-frames. The percentages of birds outside are considerably higher than those found in other studies (Dawkins et al., 2003; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). Free-range access affected the production performance of slow-growing broiler chickens. Although measured FI did not differ, IN chickens were 4 - 5% heavier at day 70, the normal slaughter age of Sasso chickens on commercial farms, than AS and SRCW chickens. This difference in weight corresponds with the findings of Castellini et al. (2002b) and Sun et al. (2013). The most likely explanation is that free-range chickens are more active and subsequently burn more calories. However, the FCR would then also be expected to be higher in the outdoor groups, which was not the case. Perhaps the actual FI in the outdoor groups was higher than the intake that we could measure, because these groups had access to additional plant material and small invertebrates found outdoors. This would mean that the real FCR would also have been higher in these groups if the calories they consumed in the free-range area would have been taken into account. Nevertheless, this data would not be relevant for farmers as they only need to track their commercial feed costs. Another explanation could be that the sample size was quite limited (n = 8 per treatment) as these variables were recorded at group level. Numerically, the FCR of the IN groups was lower than that of the AS and SRCW groups, but the greater variation in the former treatment may explain the lack of a statistical difference.

There were no significant differences in production parameters between the AS and the SRCW groups, indicating that an increased free-range use resulting from the presence of willow trees did not impact these parameters. This corresponds with Dal Bosco et al. (2014), who studied chickens with access to three different shelter types in the free-range area (none, tall grass and olive trees). It is possible that the difference in free-range use between AS and SRCW may not have been sufficient to cause considerable differences in production performance, also because the outside chickens mainly remained close to their house (Chapter 2 of this thesis).

Breast meat of AS and SRCW chickens was yellower and darker than that of the IN chickens. The increased yellowness has been reported in several other studies (Castellini et al., 2002b; Fanatico et al., 2005a; Puttaraksa et al., 2012), and could be caused by the intake of fresh plant material (*e.g.* grass or clover), which contains carotenoids (Fanatico et al., 2005a). The lighter meat colour of the IN chickens could be related to a lower pH of this meat (Allen et al., 1997; Fletcher et al., 2000), although the pH differed between AS and IN groups only.

In addition, a low pH is often related to a poorer WHC, reflected in higher drip and cooking losses. Indeed, the breast meat of IN chickens had both a lower pH and a higher drip loss than that of the AS chickens. Similar differences in pH between indoor and outdoor chickens were obtained by Ponte et al. (2008d), Jiang et al. (2011) and Almasi et al. (2015). Muscle fibre density and size may have been affected by free-range access, which could have altered the *post mortem* pH decline (Jiang et al., 2011). However, other studies found a lower pH in meat from free-range chickens than in indoor chickens (Castellini et al., 2002b; Fanatico et al., 2007; Ponte et al., 2008b; Sun et al., 2013). This is believed to be due to less pre-slaughter stress in free-range birds (because they are better able to cope with stress), which is why more glycogen remains in the muscles (Castellini et al., 2002b; Ponte et al., 2008b). Our results indicate that in the present study, muscle fibre characteristics may have been more important in determining the ultimate pH than pre-slaughter stress. However, because these factors were not taken into account in this study, we cannot be certain that this was the reason. Furthermore, the reason why AS and IN differ but SRCW and IN do not, remains unclear.

Crude fat, protein, moisture and ash did not differ between the treatment groups. It was surprising that fat content was not affected by treatment, as other studies did find lower fat content in meat from outdoor birds (Castellini et al., 2002b; Chen et al., 2013; Sun et al., 2013). Perhaps the percentage of fat in the whole carcass did differ, but this was not measured in the current study. In contrast, the fatty acid profile did show higher levels of n3, n6 and total PUFA and a higher PUFA:MUFA ratio in AS than in IN chickens. Higher levels of these fatty acids can be beneficial for human health (Dolecek, 1992; Tavazzi et al., 2008). These findings can be the result of more exercise, leading to a reduced fat content, and more or different plant intake by the AS chickens (Castellini et al., 2002b; Chen et al., 2013; Dal Bosco et al., 2016). However, other studies have shown that broiler chickens usually only eat a limited amount of pasturage (Ponte et al., 2008c; Rivera-Ferre et al., 2007a). Several individual PUFAs were higher in SRCW than in IN, but the total PUFA amount did not differ between these groups. It is known that the type and quality of the forage can influence meat composition (Ponte et al., 2008a, 2008c), so perhaps AS chickens ingested more plant material than SRCW chickens, or different plant material, as grass and clover were sown on their field, while only clover was sown on the SRCW fields. For future research it would be interesting to know the fatty acid profile of the pasture vegetation as well, so that this hypothesis can be tested.

Despite the lack of observed difference in shear force among the three treatment groups, the taste panel did score the meat from SRCW chickens as more tender and less fibrous than that of the AS and IN chickens. Preparation method may have been partly responsible for this difference (cooked in a water bath for the shear force test vs. oven-baked for the taste panel). It is also likely that the taste panel is more sensitive to changes in texture than a shear force apparatus, as this discrepancy between taste tests and shear force tests has been found in other studies (Aluwé et al., 2013; Caine et al., 2003). Regardless, meat is ultimately intended for consumers and their opinion is probably better reflected by a taste panel than by shear force testing. SRCW meat was also judged to be juicier compared to IN, although this was not reflected in the drip or cooking loss. Castellini et al. (2002b) also found juiciness to be superior in free-range chickens, even when cooking loss was also higher in this group. Taste was scored relatively low in our study for all three treatments, probably because the meat was prepared without seasoning.

Conclusions

The hypothesis of this study was that production parameters, meat quality, composition and taste would be influenced by free-range access, and that an increased use of the free-range area would lead to more pronounced differences regarding these aspects. There were indeed differences between the indoor and outdoor groups, such as a lower slaughter weight but also a better meat quality in the outdoor groups. However, the higher free-range use in the SRCW groups compared to the AS groups, generally did not cause more pronounced differences with the IN groups, although fibrousness and tenderness did differ in favour of SRCW. It is possible that the difference in free-range use was not large enough to cause a difference between AS and SRCW. To further study the relationship between meat taste and use of the outdoor run, it would be interesting to link actual use of the outdoor run of individual birds to the quality and taste of their meat.

Chapter 6

Automated positioning system

Adapted from:

L.M. Stadig, B. Ampe, T.B. Rodenburg, B. Reubens, J. Maselyne, S. Zhuang, J. Criel, and F.A.M. Tuyttens. An automated positioning system for monitoring chickens' location: accuracy and registration success in a free-range area. Submitted.

Abstract

Free-range use in chickens is often suboptimal, such that the full potential of outdoor access for chicken welfare may not be achieved. Many studies on this topic use visual observations of free-range use, imposing several limitations. An automated system capable of continuously monitoring the location of multiple individual birds over a long time period has the potential to increase the amount and accuracy of the gathered data. Therefore, the aim of this study was to test a newly developed UWB system for monitoring the position of chickens with free-range access. This system consists of active tags (attached to the chickens) that send signals to anchors positioned at fixed locations in the field; the tags' position can be calculated using the time of arrival of its signal. Its accuracy (the difference between the true position and the position registered by the UWB system) and registration success (number of registered positions divided by the number that should have been registered), as well as which factors may affect its performance, were assessed. The effects of vegetation type, precipitation, tags being mounted on a chicken, tag height, angle and orientation, coverage by A-frames or mobile chicken houses, and proximity of other tags on accuracy of the registered positions (distance between the registered and the true position of the tag) and on registration success (percentage of registrations where a position could be calculated) were assessed. Overall, the median error was 0.29 m, and the mean percentage of successful registered positions was 68%. None of the variables had a clear effect on the accuracy of the positions. Errors were generally larger in certain areas of the experimental field, which may be due to the asymmetrical setup of the anchors. The percentage of successful registrations was negatively affected by shelter type, with lower percentages in dense vegetation (short rotation coppice willows) than on grassland, possibly due to malfunctioning of two anchors close to the SRCW plots. Rain and placing the tags underneath a wooden A-frame, but not placing them in a mobile house, resulted in a lower percentage of successful registrations. The tag being mounted on a chicken, height and angle of the tag and proximity of other tags had no negative effect on the percentage of successful registrations. Placing more (functioning) anchors may contribute to better accuracy and registration success. Alternatively, the bias resulting from the variables that had a negative effect on registration success should be corrected for when using the system in its current setup. Overall, this system shows great promise to be used for monitoring chickens' free-range use.

Introduction

Chickens' free-range use, and what factors could play a role in improving it, are much studied topics (Dal Bosco et al., 2014; Fanatico et al., 2016; Pettersson et al., 2016a; Chapters 2-4 of this thesis). Most studies use visual observations by researchers to quantify free-range use, but these have several disadvantages. The presence of an observer may disturb the animals, the observations are time-consuming and often result in a limited amount of gathered data, accuracy of the data may not be optimal (e.g. it is difficult to determine the exact location of the animal in the free-range) especially when vegetation or other structures impede visibility, and it is difficult to monitor large numbers of individual animals. Studying individual animals is especially challenging in commercial situations since chickens are often kept in very large groups (thousands or tens of thousands of animals). The possibility to monitor individuals is valuable because it enables linking individual free-range use to other individual measures such as welfare, personality, and meat quality. It also enables studying differences between individuals, and underlying reasons for these differences. This way, the reasons for and effects of low or high free-range use can be studied more accurately.

An increasing number of studies are making use of automated techniques of monitoring free-range use, such as RFID, which measures if a bird is close to or crosses an antenna. The antenna is in the case of free-range studies usually placed in the pop hole so that it can monitor if the bird is inside or outside (Campbell et al., 2016b; Gebhardt-Henrich et al., 2014; Hartcher et al., 2016). Alternatively, back-mounted light sensors can be used to determine if a bird is inside or outside based on the light intensity (Buijs et al., 2017). A limitation of RFID or light sensor technologies is that they register whether the bird is inside or outside, but not its exact position. Birds can for instance remain close to the house resting, while they are recorded as using the free range. Monitoring the exact position of the birds can be used for many purposes, e.g. for calculating the distance to the house or to their closest conspecific, which can be used for social network analysis, for monitoring time spent in distinct outside areas, which can give indications of preferences for range design, or for monitoring distance travelled. Automated positioning systems (APS) have already been used in other livestock species such as dairy cows (Backman et al., 2015). Using an APS outside imposes several possible difficulties, such as the expected negative effects of water (Deak et al., 2010), meaning that e.g. rain, chickens or vegetation could hamper signal transmission.

In the current study, an APS based on UWB technology was developed in order to track chickens' position in a free-range area. This system was custom-built for the experimental field. The goal was to achieve a mean accuracy of 50 cm or better. Therefore, the aims of this study were to test accuracy and registration success under different conditions, including different shelter types on the free range (dense vegetation or grassland with artificial shelters), weather conditions (dry or rain), proximity of multiple other tags (to resemble chickens sitting close together), height and angle of the tag (to resemble different chicken positions such as sitting and standing), orientation of the tag, being covered by artificial shelters or mobile chicken houses, and the tag being worn by a chicken. These factors were studied because, if they have an effect, they could possibly result in a bias in future studies on this experimental field.

Materials and Methods

Positioning system

An UWB system was used to monitor chicken positions on an experimental field, used as a free-range area for chickens, at ILVO (Flanders, Belgium; Figure 6.1). This system works with active tags, meaning that the tags have a battery and send out a signal to receivers or "anchors". The position data is recorded and stored by the 'master' anchor (the central anchor), which sends these data to a cloud server. These anchors are placed on fixed positions on the field. Based on the time of arrival of the signal, the distance between the tag and the anchor can be calculated. If a tag's signal is received by at least three of these anchors, its position can be calculated (the intersection of the three circles that can be drawn around the anchors at that distance). We only worked with 2D positioning. The system is capable of generating Z coordinates as well, but this is more difficult because it will generate more possible intersections between the circles, or globes in this case, around the anchors. Nine anchors were placed on the experimental field (Figure 6.1), however the two anchors at the corners of the SRCW plots malfunctioned and did not contribute to the registration of tags' positions. A tag, its casing and the backpack which was used to attach it to the chicken are depicted in Figure 6.2. The effects of wearing these backpacks on the chickens' behaviour, weight gain and leg health were assessed in a separate study (Stadig et al., 2017). During all tests described here, the update rate of the tags was set at 1 Hz, i.e. a signal was sent out and a position should be registered every second. The data were recorded and stored locally using a desktop app developed specifically for this system.

Experimental field and animals

All tests were conducted on the experimental field shown in Figure 6.1, in October and November 2016. This field contained four mobile chicken houses (4.1 x 4.25 m; McGregor Polytunnels Ltd., Ropley, UK; Figure 6.3), which consisted mainly of plastic materials with an aluminium frame. The free-range areas consisted for 50% of grassland with 21 AS (wooden A-frames; l x w x h: 2.5 x 1.25 x 1.5 m; Figure 6.3), and for 50% of SRCW (Figure 6.3). SRCW was planted in 2013, in double rows, with 150 cm between two double rows, 75 cm between the two rows within a double row, and 60 cm between each tree within a row. During the time of testing the mean height of the trees was 6.6 m, and although leaf fall had commenced there were still leaves on the trees (the majority was still on in October, decreasing over time until leaf fall was completed in December). For the tests involving chickens, 42 70-day old slow-growing broiler chickens (Sasso XL451) were used that were habituated to the field. All experiments were approved by the ethical committee of the ILVO.

Accuracy and signal reception tests

To test the accuracy (i.e. the difference between the position registered by the UWB system and the true position of the tag) and registration success (in this case: the percentage of successful registrations, see 2.5) of the system, tags were placed at fixed positions on the field in different configurations, depending on what was being tested. Table 6.1 gives an overview of all situations that were tested. Most tests were repeated for all four 'subfields' (the triangular fields in Figure 6.1, with a mobile house in their centre), identified by the number of the mobile house on that subfield. All tests lasted for 1 minute. The locations that were used for the tests are shown in Figure 6.1. For the 'straight line' tests the tags were positioned 5 m apart from each other on the boundary of grass and SRCW, and 1 m towards each side onto the grass and the SRCW. This was done because we wanted to know how well chickens would be detected on this border between grass and SRCW, in order to perform shelter type preference tests in the future. For the 'diagonal line' tests the tags were placed on two diagonal lines (one on grassland, one in SRCW) which stretched between the central corner and the edge of the subfield. This was done in order to test the effect of shelter type. This test was repeated with the tags on the grass being covered by A-frames. For the tests located '10 cm from wall of house' tags were placed at each corner and in the middle of each side of the house, both indoors and outdoors (all 10 cm from the wall). The same was done for the '30 cm from wall of house' tests, but then

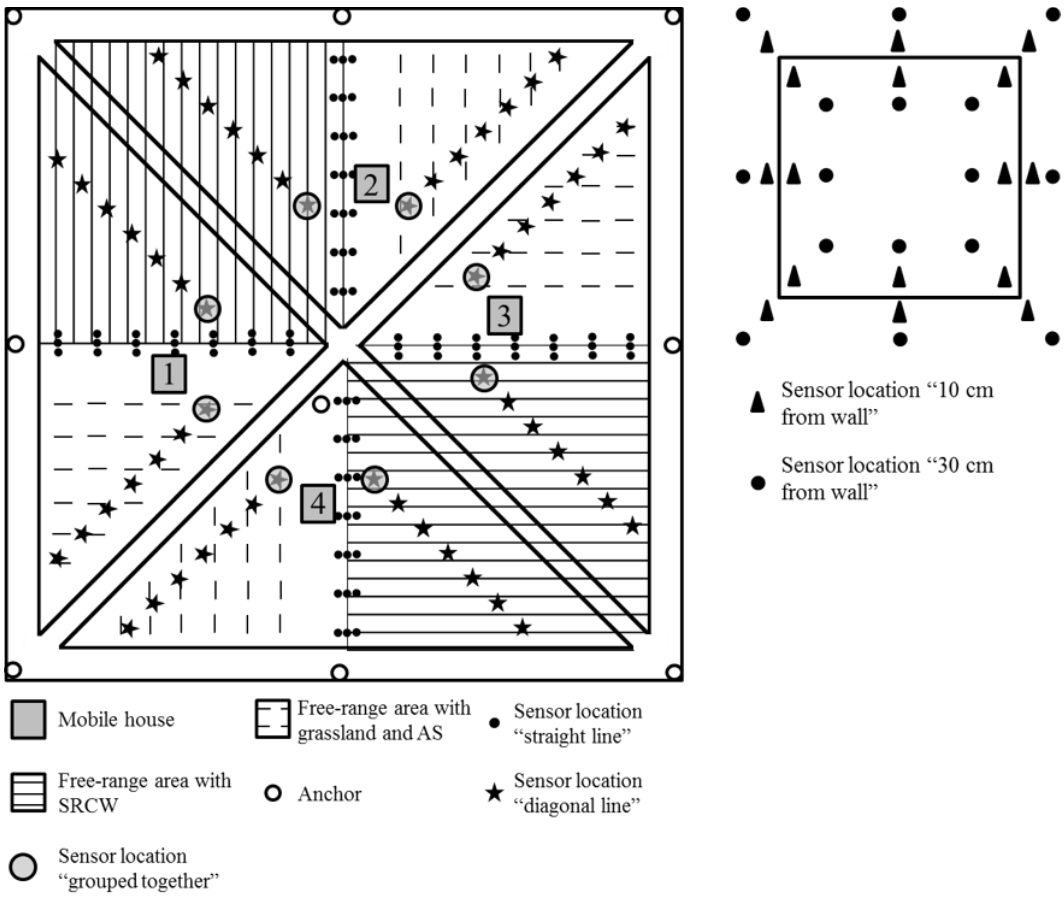


Figure 6.1 Left: Overview of the experimental field (outer boundaries: 100 x 100 m) and the positions of the tags during the "straight line", "diagonal line" and "grouped together" tests. AS = artificial shelter, SRCW = short rotation coppice willows. The anchors in the upper left and lower right corners did not function. Right: Outline of one mobile house with an overview of the positions of the tags relative to the house during the "10 cm from wall" and "30 cm from wall" tests.

Figure 6.2 From left to right: a tag, its casing, the backpack, and a chicken with the backpack. The weight of tag and its casing is 36 g.

Figure 6.3 Left: grassland with artificial shelter (AS). Middle: short rotation coppice willows (SRCW). Right: mobile chicken house.

with tags placed 30 cm from the walls (Figure 6.1). This was done to test if the proximity of a mobile house would influence the system's performance, taking into account that chickens spend much time in on just outside the house. For the 'grouped together' tests, all tags were placed together (ca. 5 cm apart), once on the grass, once in the SRCW (both at the position on the diagonal line closest to the central corner of the triangular subfield) and once in the centre of the house. This was done because chickens often flock together, and it was unknown if many tags in close proximity of each other would influence accuracy or registration success.

For the tests without chickens, the tags (in their casing and backpack) were placed on plastic boxes, which were subsequently placed on known positions on the field. The boxes' dimensions were 12.5 x 20 x 34 cm, so that the tags could be placed at both 12.5 and 20 cm height, to resemble chickens at different heights. First, all boxes were levelled and the tags were placed flat on top of them for the measurements at 0° angle, to create a 30° angle with the horizontal plane a small wooden block was placed underneath the tag. These angles were chosen because they were assumed to be the most likely to occur if the tags would be back-mounted on a chicken. It was also tested if the orientation of the tag influenced the measurements, by testing four different orientations of the antenna (Figure 6.4). In the other tests, the antenna of the tag was always directed inwards.

For the tests with chickens, the backpacks with the tags were attached to the chickens. These were subsequently placed in a cardboard box so they could be placed on fixed positions on the field. To test if the cardboard box itself had an influence on the measurements, an additional test was done in which cardboard boxes were placed over the tags on the middle line of the 'straight line' tests during the tests without chickens. Tests with chickens were only performed for subfields 2 and 4, since it was expected that being contained in the box, in social isolation,

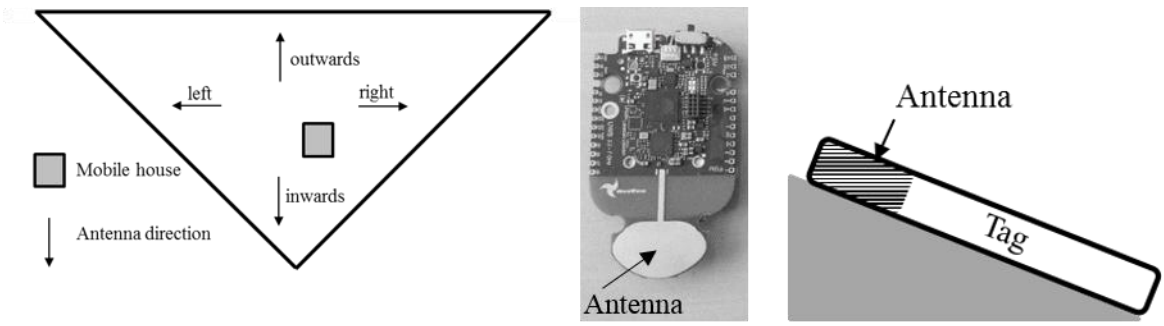


Figure 6.4 Left: Tested antenna directions depicted on one subfield (top view). Middle: Tag with its antenna. Right: side view of the tag under a 30° angle.

could be quite stressful for the birds, especially in warm weather conditions. Therefore, we wanted to keep the number of animals exposed to this stress limited. It was reasoned that testing two of the subfields would be sufficient since no effects of 'subfield' on the accuracy or registration success were expected.

Calculating accuracy

All positions on the field that were used to place the tags were also measured using a precision-GPS device (S10 GNSS Receiver, Stonex, Monza, Italy). This device had a reported horizontal precision of 3 mm. All GPS measurements in the SRCW plots were done in the winter when no leaves were present, because those may impede GPS accuracy. To calculate the observed accuracy of the UWB system, the positions registered by that system were compared with the positions of the GPS device.

Calculating signal reception

Per test, it was counted how many registrations there were for each position of the tag. As the update rate was set at 1 Hz, and the tests lasted 1 minute, it was expected that each tag would be registered 60 times per test. However, it was noted that the registrations only started several seconds after the start of each test due to a delay in the desktop app. Therefore, the maximum possible number of registrations per test was determined by the tag with the highest number of registrations by at least one anchor in that particular test. Then, per tag, the number of successful registrations (successful meaning that there was a position estimate, so at least three anchors picked up the tag signal) was divided by the maximum possible number of that test and multiplied by a hundred to obtain the percentage of successful registrations per tag per test. In order to assess the effects of vegetation, rain, chicken presence, height, angle, orientation, coverage by A-frames or mobile houses, and proximity of other tags, the average percentage successful registrations for that particular research question was calculated.

Data analysis

Table 6.2 shows which data were used to answer each of the research questions. For each research question, the median and mean errors were calculated, as well as the percentages of registrations with an error below 0.5 m (the requested accuracy), 1 m (large errors) and 10 m (very large errors). In addition, the mean percentage of successful registrations were calculated, split up into the categories to be tested for that particular question. For example, to test for the effect of rain, the results were split up into those of dry and wet days. Because there sometimes were considerable differences between the four subfields, accuracy and successful registrations were calculated separately for each subfield, as well as for the entire field.

Results

Overall, the median error was 0.29 m, and the mean percentage of successful registration was 68%. The results showed considerable differences in accuracy and percentage successful registrations between the four subfields. In general, the errors were smallest on subfields 4 and 1, and highest on subfield 2 (Table 6.3). Mean percentage of successful registrations was lowest on subfield 3, and highest on subfields 4 and 1 (Table 6.3).

Effects of vegetation, rain and chicken presence

No clear differences in the median error between the different vegetation types (SRCW, grass and the boundary between these) were observed (Table 6.4). The higher mean errors on subfield 3 for SRCW and AS were mainly due to large errors on the 'diagonal lines'; if only the 'straight lines' were taken into account mean errors were 0.34 m, 0.32 m and 1.23 m for SRCW, boundary and grass, respectively (data not shown). Percentage of successful registrations was 16-20% lower in SRCW than on grassland and on the boundary. The median error was not affected by rain (Table 6.4). Percentage of successful registrations was 12% lower on days with rain. When looking at the separate subfields, this measure did appear to be only influenced by rain on subfields 1 and 3, not on subfields 2 and 4. The median and mean errors on subfield 2 were considerably higher with the tags being mounted on a chicken than without chickens (Table 6.4). However, this was not the case on subfield 4. Percentage of successful registrations did not appear to be influenced by chicken presence.

Effects of height, angle and orientation of the tag

Placing the tags at a height of 20 cm compared to 12.5 cm did not influence the median error or percentage of successful registrations (Table 6.4). The tag being under a 30° angle neither influenced the median error, nor did percentage of successful registrations appear to be influenced by angle of the tag. Orientation of the tag (direction of the antenna) did not influence the median or mean error. Percentage of successful registrations was lowest when the antenna was directed inwards, and highest when it was directed outwards.

Effects of A-frames, mobile houses, cardboard boxes and proximity of other tags

Being covered by a wooden A-frame did not affect the median or mean error. Overall, a 13%-decrease in percentage of successful registrations was observed, although this effect was not observed in subfield 4 (Table 6.4). Being covered by a mobile house also did not affect median or mean error or percentage of successful registrations. Cardboard boxes had no effects on median or mean error, or percentage of successful registrations. The proximity of other tags did not negatively influence mean or median error, nor did it affect percentage successful registrations (Table 6.4).

Table 6.1 Overview of all measurements performed to test which factors affected accuracy and signal reception of the automated positioning system. Also see Figures 6.1 and 6.4 for more information on the location, angle and orientation of the tags.

	Specifications for measurements with chickens		Measurements with chickens				Specifications for measurements without chickens				Measurements without chickens - dry day				Measurements without chickens - rainy day			
			Subfield								Subfield				Subfield			
Location	Orientations (1 or 4)	Extra comment	1	2	3	4	Height (cm)	Angle (0 or 30 ° from horizontal plane)	Orientati-ons (1 or 4)	Extra comment	1	2	3	4	1	2	3	4
• Straight line	4		-	+	-	+	12.5	0	1[a]		+	+	+	+	+	+	+	+
							12.5	30	1		+	+	+	+	+	+	+	+
							20	0	1		+	+	+	+	+	+	+	+
							20	30	4		+	+	+	+	+[b]	+[b]	+[b]	+[b]
							12.5	30	1	I	+	+	+	+	-	-	-	-
★ Diagonal line	4		-	+	-	+	12.5	0	1		+	+	+	+	+	+	+	+
	1	II	-	+	-	+	12.5	30	1		+	+	+	+	+	+	+	+
							20	0	1		+	+	+	+	+	+	+	+
							20	30	4		+	+	+	+	+[b]	+[b]	+[b]	+[b]
							20	30	1	II	+	+	+	+	+	+	+	+
10 cm from wall of house	4		-	+	-	+	12.5	0	1		+	+	+	+	+	+	+	+
							12.5	30	4		+	+	+	+	+[b]	+[b]	+[b]	+[b]
30 cm from wall of house	4		-	+	-	+	12.5	0	1		+	+	+	+	+	+	+	+
							12.5	30	4		+	+	+	+	+[b]	+[b]	+[b]	+[b]
○ Grouped together on grass	1		-	+	-	+	12.5	0	1		+	+	+	+	-	-	-	-
○ Grouped together in SRCW	1		-	+	-	+	12.5	0	1		+	+	+	+	-	-	-	-
○ Grouped together in house	1		-	+	-	+	12.5	0	1		+	+	+	+	-	-	-	-

○ • ★ *Correspond with the symbols in Figure 1. + = was included in test, - = was not included in test.* [a] *if only one orientation was tested, this was the 'inwards' orientation;* [b] *only one orientation; I = with cardboard box over eight tags on the grass/SRCW boundary; II = with A-frames over tags on grassland.*

Table 6.2 Overview of which tests were used to test for effects of shelter type, rain, chicken presence, height, angle, orientation. Variables in bold indicate that the dataset was split up according to this variable to answer this specific research question.

Effect of?	Chickens	Subfield	Weather condi-tion(s)	Location(s)	Vegetation/ shelter	Height(s) (cm)	Angle	Orientation	Tags covered by A-frames	Nr of tags per position	Tag covered by box
Vegetation	No	1, 2, 3, 4	Dry	Straight line Diagonal line	**SRCW Boundary Grass**	12.5, 20	0, 30	Antenna directed inwards	No	1	No
Rain	No	1, 2, 3, 4	**Dry, Rain**	Straight line, Diagonal line, 10/30 cm from wall	All	12.5, 20	0, 30	Antenna directed inwards	No	1	No
Chicken presence	**No, Yes**	2, 4	Dry	Straight line, Diagonal line, 10/30 cm from wall	All	12.5, 20[1]	0, 30[1]	Antenna directed inwards	No	1	No, Yes
Height	No	1, 2, 3, 4	Dry	Straight line, Diagonal line	All	**12.5, 20**	0, 30	Antenna directed inwards	No	1	No
Angle	No	1, 2, 3, 4	Dry	Straight line, Diagonal line, 10/30 cm from wall	All	12.5, 20	**0, 30**	Antenna directed inwards	No	1	No
Orientation	No	1, 2, 3, 4	Dry	Straight line, Diagonal line	All	20	30	**Antenna in four different directions**	No	1	No
A-frames	No	1, 2, 3, 4	Dry	Diagonal line on grassland	Grass	20	30	Antenna directed to the right	**No, Yes**	1	No
Proximity of other tags	No	1, 2, 3, 4	Dry	1 position	SRCW, Grass, Mobile house	12.5	0	Antenna directed inwards	No	**1, 21**	No
Coverage by mobile house	No	1, 2, 3, 4	Dry	10/30 cm from wall	**Inside house, Outside house**	12.5, 20	0, 30	Antenna directed inwards	No	1	No
Coverage by cardboard box	No	1, 2, 3, 4	Dry	Straight line	Boundary	12.5	30	Antenna directed inwards	No	1	**No, Yes**

[1] *Only applies to measurements without chickens*

Table 6.3 Median and mean errors, percentage of measurements with an error below 1 m, 0.7 m, 0.5 m and 0.3 m (n = 142,944 registered positions) and mean successful registrations (n = 3,426; 208 tests with 7 - 24 tags per test) per subfield and for the entire field.

	Subfield 1	Subfield 2	Subfield 3	Subfield 4	Entire field
Median error (m)	0.30	0.37	0.29	0.25	0.29
Mean error (m)	0.42	2.86	1.14	0.30	1.12
Error (m)					
< 10	100%	91%	97%	100%	97%
< 1	97%	74%	92%	99%	91%
< 0.7	96%	69%	88%	96%	88%
< 0.5	90%	60%	81%	88%	80%
< 0.3	51%	42%	52%	59%	52%
Mean percentage of successful registrations	76%	73%	43%	77%	68%

Discussion

Overall, the accuracy of the UWB system tested in this study met our goal; the median error was 29 cm and an error of less than 50 cm was achieved in the majority of the measurements, although there were clear differences between the four subfields (percentages of positions with an error <50 cm varying between 60 and 90%). The very large errors (>10 m) that occurred in some tests such as those with chickens on subfield 2 did not seem to be related to particular circumstances such as rain or being in the SRCW. This is important because it shows that these variables do not influence the accuracy of this system. The errors were not due to a low number of anchors registering the tags, or to the presence of several intersections between the circles from the different anchors far apart from each other. In other studies with UWB systems, large errors are found in proximity of metal-containing buildings (MacGougan et al., 2009), but such buildings were not present around our experimental field. The reason for the difference between the subfields may be the setup of the anchors. They were not distributed exactly symmetrically over the field, because the central anchor could not be positioned central on the field due to practical reasons. Therefore, it was positioned closer to subfields 1 and 4 than to subfield 2 and 3. This may explain why most of the large errors occurred in the latter two subfields. The line of sight (no obstructions between the tag and the anchor) between the central anchor and tags on the former two subfields may have been better. The absence of line of sight could have resulted in occasional large errors on subfields 2 and 3, especially on the latter where the likelihood of having a line of sight will be the lowest. The signal may travel an alternative path

Table 6.4 Median and mean error, percentage of errors below 0.5, 1 and 10 m, and percentage of successful registrations per tested parameter. SRCW = short rotation coppice willows.

Effect of?		Subfield	Median error (m)	Mean error (m)	Percentage of registrations with error below			No. of registered positions	Successful registrations (%)	No. of tags in tests[1]
					0.5 m	1 m	10 m			
Vegetation	SRCW	1	0.32	0.35	89	97	100	1990	66	56
		2	0.25	0.29	90	100	100	1018	34	56
		3	0.30	3.69	79	88	89	1155	34	60
		4	0.23	0.24	97	100	100	2332	45	52
		Entire field	0.26	0.90	90	97	98	6495	45	224
	Boundary	1	0.38	0.44	80	97	100	1461	97	28
		2	0.52	0.59	45	87	100	1203	79	28
		3	0.25	0.32	96	99	100	629	39	32
		4	0.26	0.31	84	99	100	1927	49	28
		Entire field	0.35	0.41	75	96	100	5220	65	116
	Grass	1	0.30	0.39	89	94	100	2209	79	52
		2	0.25	0.34	87	94	100	1677	55	56
		3	0.35	4.39	64	68	88	1533	42	64
		4	0.23	0.28	89	99	100	4592	73	56
		Entire field	0.27	0.94	85	92	98	10011	61	228
Rain	No	1	0.30	0.36	89	97	100	8813	82	200
		2	0.28	0.36	78	96	100	7148	65	204
		3	0.30	2.55	77	83	93	4605	39	220
		4	0.24	0.27	92	99	100	12551	70	200
		Entire field	0.27	0.63	86	96	99	33117	63	824
	Yes	1	0.33	0.75	87	95	98	6031	50	200
		2	0.25	0.35	80	94	100	6796	64	204
		3	0.22	1.06	81	90	93	2832	25	220
		4	0.21	0.26	95	99	100	10682	72	200
		Entire field	0.25	0.48	88	96	99	26341	51	824
Chicken presence	No	2	0.28	0.36	78	96	100	7148	65	204
		4	0.24	0.27	92	99	100	12551	70	200
		Entire field	0.25	0.30	87	98	100	19699	68	404
	Yes	2	5.05	10.54	2	5	64	2173	71	57
		4	0.25	0.30	86	100	100	3294	72	66
		Entire field	0.44	4.37	53	62	86	5467	72	123

Effect of?		Subfield	Median error (m)	Mean error (m)	Percentage of registrations with error below			No. of registered positions	Successful registrations (%)	No. of tags in tests
					0.5 m	1 m	10 m			
Height	12.5 cm	1	0.33	0.45	81	93	100	2649	73	68
		2	0.34	0.44	90	90	100	1870	49	70
		3	0.33	5.61	82	75	83	1850	39	78
		4	0.22	0.28	90	99	100	3522	56	68
		Entire field	0.30	1.35	80	91	100	9891	54	284
	20 cm	1	0.32	0.33	92	98	100	3011	82	68
		2	0.30	0.37	80	96	100	2028	54	70
		3	0.28	0.56	80	89	100	1467	38	78
		4	0.24	0.27	89	100	100	5329	58	68
		Entire field	0.27	0.34	87	97	100	11835	57	284
Angle	0°	1	0.28	0.37	86	95	100	4445	81	100
		2	0.33	0.41	71	95	100	3521	66	102
		3	0.27	4.97	69	74	85	2116	42	110
		4	0.22	0.26	93	99	100	5628	71	100
		Entire field	0.27	0.96	83	94	98	15710	62	412
	30°	1	0.32	0.35	92	98	100	4368	82	100
		2	0.21	0.30	84	97	100	3627	65	102
		3	0.32	0.49	84	92	100	2489	35	110
		4	0.24	0.28	91	99	100	6923	69	100
		Entire field	0.27	0.33	89	97	100	17407	64	412
Covered by A-frames	No	1	0.30	0.28	100	100	100	293	96	6
		2	0.14	0.18	100	100	100	323	85	7
		3	0.41	0.40	92	97	100	306	72	8
		4	0.33	0.28	100	100	100	309	80	7
		Entire field	0.28	0.28	98	99	100	1231	82	28
	Yes	1	0.24	0.24	100	100	100	260	82	6
		2	0.16	0.19	100	100	100	257	69	7
		3	0.39	0.38	93	99	100	197	46	8
		4	0.28	0.26	99	100	100	312	84	7
		Entire field	0.25	0.26	99	100	100	1026	69	28

Effect of?		Subfield	Median error (m)	Mean error (m)	Percentage of registrations with error below			No. of registered positions	Successful registrations (%)	No. of tags in tests
					0.5 m	1 m	10 m			
Orientation	Outwards	1	0.28	0.36	92	96	100	1622	90	34
		2	0.21	0.33	86	96	100	1475	80	35
		3	0.31	0.81	81	92	98	1502	71	39
		4	0.27	0.32	92	100	100	1869	68	34
		Entire field	0.27	0.44	88	96	99	6468	77	142
	Right	1	0.30	0.29	99	100	100	1481	87	34
		2	0.23	0.39	78	93	100	1291	68	35
		3	0.36	0.46	81	98	100	1100	52	39
		4	0.24	0.34	85	98	100	2001	67	34
		Entire field	0.29	0.36	86	97	100	5873	68	142
	Inwards	1	0.33	0.32	95	100	100	1522	83	34
		2	0.30	0.36	79	97	100	1032	54	35
		3	0.32	0.58	80	90	100	952	46	39
		4	0.26	0.29	87	100	100	3257	58	34
		Entire field	0.29	0.35	87	98	100	6763	60	142
	Left	1	0.28	0.37	87	96	100	1555	87	34
		2	0.18	0.24	91	100	100	1249	67	35
		3	0.28	0.30	90	99	100	1253	60	39
		4	0.27	0.29	87	100	100	3343	61	34
		Entire field	0.26	0.30	88	99	100	7400	69	142
Covered by mobile house	No	1	0.24	0.33	89	97	100	1613	93	32
		2	0.22	0.29	83	100	100	1565	92	32
		3	0.25	0.30	91	100	100	311	19	32
		4	0.25	0.29	94	99	100	1823	95	32
		Entire field	0.24	0.31	89	99	100	5312	86	128
	Yes	1	0.25	0.26	98	99	100	1540	88	32
		2	0.23	0.31	80	100	100	1685	99	32
		3	0.29	0.48	77	86	100	977	59	32
		4	0.22	0.25	98	100	100	1877	98	32
		Entire field	0.24	0.30	90	98	100	6079	86	128

Effect of?		Subfield	Median error	Mean error (m)	Percentage of registrations with error below			No. of registered positions	Successful registrations (%)	No. of tags in tests
					0.5 m	1 m	10 m			
Covered by cardboard box	No	1	0.37	0.38	83	99	100	461	97	9
		2	0.42	0.51	60	85	100	418	86	9
		3	0.34	0.89	79	80	100	257	47	10
		4	0.20	0.24	94	100	100	574	60	9
		Entire field	0.32	0.44	81	93	100	1710	72	37
	Yes	1	0.39	0.38	80	100	100	367	99	7
		2	0.54	0.60	39	83	100	320	83	7
		3	0.33	0.30	91	100	100	146	42	7
		4	0.22	0.25	90	100	100	368	97	7
		Entire field	0.34	0.39	74	95	100	1201	80	28
Proximity of other tags	No	1	0.30	0.30	99	100	100	101	99	2
		2	0.12	0.12	100	100	100	55	48	2
		3	0.17	6.47	53	53	53	90	55	2
		4	0.15	0.20	99	100	100	129	98	2
		Entire field	0.25	1.72	88	88	89	375	75	8
	Yes	1	0.32	0.34	78	99	100	3307	97	63
		2	0.37	0.42	72	98	100	3010	89	63
		3	0.29	0.40	82	98	100	1945	50	63
		4	0.28	0.31	89	100	100	3372	98	63
		Entire field	0.31	0.36	80	99	100	11634	82	261

[1] *Differences between subfields are due to different numbers of tags used. All lines in the 'straight line' and 'diagonal line' test contained seven tags, except for the diagonal line on grass on subfield 1 (six tags), the straight line and diagonal line on grass on subfield 3 (both eight tags), and the diagonal line in SRCW on subfield 4 (six tags). This was due to the field and configuration of the tags not being completely symmetrical, leading to somewhat shorter or longer lines in some cases.*

(non-line of sight signal), leading to a wrong distance to that anchor being recorded (Yang et al., 2013). The central anchor is not essential for registration of the position, but may be needed if other anchors around the field fail to receive the signal. In addition, it is better for the signal to be received by anchors on several places around the tag, instead of by anchors that are all aligned, because such an alignment often gives more options for the circles to intersect.

When this system were to be used for monitoring chickens' positions, it is important to be able to filter out faulty registrations. There are several options to do this. With the algorithm that was used, it was noticed that if the standard error of the estimation of the position was larger than 100 m, the position was always outside of the field that the tag was in, so these recordings could be recognised as 'wrong'. Similarly, all registrations with a position outside of the area where the chickens could be present could be deleted (at night: outside the house; during the day: outside of the free-range area). However, these methods do not discard all faulty registrations, and this is an aspect to investigate further. Another option could be the use of 'smoothers', but these require a sufficiently high sampling rate. For example, if the sampling rate is 1 Hz, and there is 50 m between one registration and the next, while the following registration is close to the first one, the middle observation can be considered an outlier and corrected to a position in between the first and third observation. In this case, a clear definition of 'impossible' position registrations is needed (e.g. should this be movements of over 10 m/s or 50 m/s).

To our knowledge, this UWB system is the first APS capable of accurately monitoring positions of individual chickens kept in groups in an outdoor area. GPS technology has been used by Dal Bosco et al. (2010), but no accuracy tests were done in that study. According to the manufacturer, their system had an accuracy of 2.5 m. Taking into account that broiler chickens prefer to stay close to their house (Dawkins et al., 2003), an error of 2.5 m (which implies a buffer zone of 2.5 m around the house should be taken into account in order to say if a bird was truly outside or not) means a possible under- or overestimation of the number of chickens outside. An APS developed and tested by Quwaider et al. (2010) in an indoor environment for laying hens was reported to have 84% agreement between the sensor system and video observations. Agreement was defined as both the video observer and the APS registering the tag being within 1 m of a receiver, which were close to resources such as feeders and nest boxes. However, that system only made use of relative localization, i.e. the tag registered its distance to the receivers but no absolute localization was performed. Therefore, accuracy as it is reported in the current study was not determined.

For the UWB system used in the present study, vegetation type (SRCW, grassland or the boundary in between) had no effect on the accuracy. However, percentage successful registrations was 16 – 20% lower in SRCW than on grassland. This could be due to the signal being absorbed by water in the leaves, by the trees blocking the line of sight between the tags and the anchors, or by the two anchors at the corners of the SRCW plots that did not function. This means that if the system would be used to test birds' preference between these shelter types, the data could be biased with an undervaluation of the birds in SRCW. Therefore, it is important to examine the setup and the proper functioning of the anchors. When it was simulated that the anchor at the corner of the grassland next to subfields 1 and 4, or alternatively, 2 and 3 was not functioning (by excluding these data from the dataset), this resulted in a decrease of registered positions on the grassland between 9 and 19% (data not shown). This shows that it is likely that the non-functioning anchors at the corners of the SRCW plots were at least partly responsible for the missing data. It therefore needs to be tested whether replacing these anchors, or alternatively placing more anchors on/around the SRCW plots, improves the percentage of successful registrations. Alternatively, it may be possible to correct for the bias resulting from a higher percentage of successful registrations on grassland. For example, depending on the sampling rate, it may be reasonable to assume that if a certain number of observations are missing, and those before and after the gap show the same location, the animal was in that place during the missing observations as well. Or, the number of registered positions in SRCW could be multiplied by the estimated percentage of missed locations, and the same could be done for the grassland.

Rain had no negative impact on the accuracy of the system, but the percentage of successful registrations was on average 12% lower on rainy than on dry days. This could be due to the water absorbing the signal. Rain is something that cannot be avoided in free-range studies, especially because weather conditions, including rainfall, play an important role in free-range use (Chapters 2 and 3 of this thesis). It was not tested from which level of rainfall the percentage of successful registrations started to decrease. It may be that this only starts to decrease at a level of rainfall at which the chickens will mainly seek shelter in their houses, as free-range use decreases with rainfall use (Chapters 2 and 3 of this thesis), but this remains to be tested.

The tag being mounted on a chicken seemed to increase the error considerably; when looking at subfield 2 this was indeed the case, but not in subfield 4. It may therefore be carefully assumed that the reason for the large errors in subfield 2 is possibly another than the presence of the chicken, although it is not known what this reason is. The percentage of successful registrations was not reduced when the tag was worn by a chicken compared to when it was not, which means that data gathered without chickens is representative for those gathered with tags mounted to the animals. The cardboard boxes that contained the chickens during the tests had no effects on accuracy or percentage of successful registrations, so it can be concluded that any differences between measurements with and without chickens cannot be attributed to these. This implies that testing an UWB system can be done without chickens (in the current setup), by simply putting the tags at fixed positions on the field. This makes testing easier and reduces the number of animals used. In addition to the chickens not influencing the position registration, it is also important for future use of the APS that the tags do not influence the behaviour and welfare of the birds. To test this, a study was performed in which the behaviour, weight gain and leg health were monitored of slow-growing broilers (Sasso XL451). These were either mounted with a backpack with a tag or colour-marked with spray paint for individual identification. Results from this study show no effects of wearing the backpack after the first week the birds were fitted with them, and no effects on weight gain or leg health (Stadig et al., 2017).

Neither accuracy nor the likelihood of missed registrations were influenced by the height of the tags. This means that the size or posture (standing or sitting) of the chickens are not likely to have an influence on the likelihood of a position being registered or on the positions' accuracy. This UWB system also provides Z coordinates, so in theory the height of the animals can also be registered, although as mentioned in the methods sections this may not be straightforward. In our studies this was not relevant since the birds were floor-housed and did not have access to elevated structures, but if the system were to be used for e.g. laying hens with aviary housing or elevated platforms or nest boxes, this option could prove to be useful.

The angle of the tag and its antenna did not influence accuracy or percentage of successful registrations. The lack of difference between the different angles of the antenna indicates that if the tag is on a chicken, accuracy and registration success will not be influenced if the angles changes, at least between a level position and a 30° angle. The orientation of the tag had no major impact on the mean or median errors of the registered positions. However, percentage of successful registrations was lowest if the antenna was directed inwards, and highest if it was directed outwards. This could have implications for when the system will be used on chickens in the future, since their direction is not known. It is therefore important to further investigate what caused this difference and how it can be remedied.

If tags were covered by an A-frame this did not influence the accuracy of the registered positions, but it did negatively affect the registration success, with an overall reduction of 13%. The mobile houses did not negatively impact accuracy, and neither had an effect on percentage successful registrations. This discrepancy compared to the results from the A-frames could be related to the materials both were made of; the A-frames were constructed of shuttered plywood while the houses were mainly plastic with an aluminium frame. The finding that mobile houses had no influence on accuracy or registration success is important, otherwise a bias could exist for registering chickens' positions if they were inside or outside.

Proximity of other tags could be a challenge due to multi-user interference. However, in this study it had no negative impact on the accuracy of registered positions nor on the percentage of successful registrations. Since chickens often tend to flock together, it is important that the proximity of other tags does not interfere with the quality of the measurements, which does not seem to be the case with the UWB technology used in the present study. This indicates that the system is suitable for monitoring many individuals at high density simultaneously, at least up to 21 tags which was the number of tags used in these tests.

Conclusions

This UWB system shows potential for automated and simultaneous registration of the location of many individual chickens with free-range access. Even with two malfunctioning anchors, the vast majority (60 – 90%) of the measurements had an error below 50 cm. None of the tested factors had a clear negative effect on the accuracy. There were some very large errors (>10 m) and the lowest percentage of successful registrations was 19%; possibly due to an asymmetrical setup of the anchors leading to poorer registration success in subfields 2 and 3. The percentage of successful registrations was negatively influenced by dense vegetation, by coverage of tags by A-frames, and by rain. The lower percentage of successful registrations in SRCW was probably at least partially due to the two anchors that were not working, and would otherwise have likely been considerably higher. To further optimize the accuracy and registration success it is important that all anchors are working correctly, and it may be worth re-examining the setup of the anchors to assess if more anchors need to be placed on the field. Other factors, such as tags being mounted on a chicken, height and angle of the tag, coverage of mobile houses and proximity of other tags had no effect on registration success. Overall, the results are promising and the system appears suitable for monitoring the positions of chickens with free-range access.

Chapter 7

Interactions between broilers, SRCW and soil parameters

Adapted from:

L.M. Stadig, F.A.M. Tuyttens, T.B. Rodenburg, B. Vandecasteele, B. Ampe, and B. Reubens. Interactions between broiler chickens, soil parameters and short rotation coppice willow in a free-range system. Submitted.

Abstract

Free-range areas for poultry often consist of grassland. Planting woody vegetation such as SRCW in these areas could have several advantages: promoting free-range use resulting from suitable shelter, a higher extent of multilevel land use through productive and regulating ecosystem services, and increasing biodiversity. The aim of this study was to test the effects of combining SRCW and chickens on free-range use, soil conditions and SRCW growth. A 1-ha field was split up into four quadrants: two were sown with a grass/clover mixture, two were planted with SRCW (three clones, i.e. Tora, Tordis and Klara). SRCW was harvested 1 and 4 years after establishment. Chickens were present on the field during parts of each year, and parts of the field were kept chicken-free as a control. Free-range use, SRCW growth and soil parameters were monitored on a regular basis. Chickens preferred to range in SRCW compared to grassland. No effects of chicken presence on SRCW growth were observed. Total mineral N (N_{min}) was affected by vegetation type x location x depth; it was generally higher in SRCW than in grassland, in areas close to the chicken houses, and in more superficial soil layers. This could be due to return of N through leaf fall (as opposed to grass which is mown and removed), to the higher chicken density in SRCW (more N deposition through faeces), to NH_3 being captured from the air by the trees, to the strong clover development under SRCW (which can fix atmospheric N), and to the lower N requirement of SRCW compared to grassland. N_{min} did not appear to accumulate in the soil over the years, but there were strong indications for higher risk of nitrate leaching to deeper soil layers and possibly to groundwater close to the houses and in SRCW. K and P-$CaCl_2$ were higher close to the chicken houses, probably due to high concentrations of these nutrients in chicken faeces. No difference in soil organic carbon was observed in SRCW compared to grassland, this could be due to the short time period that SRCW was present. In conclusion, SRCW was beneficial for the chickens, but the combination needs to be studied further, and possible remediating strategies need to be tested in order to prevent nitrate and P from leaching to groundwater.

Introduction

In the EU, for organic and most free-range laying hens and broiler (meat-type) chickens, it is required to provide at least 4 m^2 outdoor space per animal (European Commission, 2008b). Taking into account that the average organic or free-range chicken farm houses tens of thousands of chickens (Stadig et al., 2016), the average free-range area consists of multiple hectares. This area often consists mainly of grassland, which is relatively easy for the farmer to manage, but has several disadvantages when compared to e.g. woody vegetation. First, chickens originate from jungle fowl whose natural habitat is dense vegetation. Modern-day domestic chickens make limited use of the free-range area (Dawkins et al., 2003; Hegelund et al., 2005; Jones et al., 2007; Pettersson et al., 2016a) if no shelter against predators or adverse weather conditions is provided. EU legislation states that organic free-range areas should be "mainly covered with vegetation and be provided with protective facilities" (European Commission, 2008b). Increased free-range use could benefit animal welfare, and also reduce point sources of pollution which result from concentration of chickens close to the chicken house. Second, other (woody) types of vegetation might contribute to a higher extent of multilevel land use, through the delivery of a set of productive (e.g. renewable energy, wood, fruit or nut production, etc.) or regulating ecosystem services (e.g. carbon storage, improved water or air quality by decreased nitrogen (N) leaching or enhanced N entrapment, respectively) and through increasing biodiversity.

A vegetation that could possibly achieve several of the benefits mentioned above, is SRCW. This is a fast-growing energy crop that can be harvested every three years (Caslin et al., 2010). SRCW can produce between 10 and 16 x 10^3 kg dry matter/ha/year (Albertsson et al., 2016; Bergante et al., 2016; Stolarski et al., 2013), which makes it a suitable source for renewable energy production. Several studies showed that willows have the potential for soil C sequestration (Dimitriou et al., 2012b; Grogan and Matthews, 2002). In addition, they can decrease the risk of N leaching (Dimitriou et al., 2012a; Goodlass et al., 2007), due to their ability to take up water and N from deeper soil layers (Bergström and Johansson, 1992) and can have a positive legacy effect on soil quality and subsequent crops (Schrama et al., 2016). This is potentially very relevant in a free-range context, because chicken excreta contain high amounts of N and P, which can cause too high N and P concentrations in the soil, especially at locations where chicken density is high (Häne et al., 2000; Kratz et al., 2004; Zeltner and Hirt, 2008). Apart from taking up N and P from the soil, the SRCW can also help to decrease point source of pollution by promoting a better distribution of the flock over the entire free-range area, through the provision of shelter against threats to the chickens which therefore may range further from the house (Chapters 2-4 of this thesis). Finally, the presence of SRCW has also been observed to be related with an increased biodiversity both aboveground and belowground (Baum et al., 2009; Langeveld et al., 2012; Rowe et al., 2011; Sage, 1998; Schrama et al., 2016).

Despite the promising benefits of SRCW, research about the performance of this crop in the context of chickens' free-range areas is currently lacking. Therefore, the aim of the present study is to investigate 1) how the presence of chickens influences soil conditions (hypothesis: higher nutrient concentrations and more risk for N leaching in areas with high chicken density), 2) how the presence of chickens influences SRCW growth (hypothesis: increased growth due to increased supply of nutrients through faeces), and 3) how SRCW influences soil conditions as compared to grassland (hypotheses: increased C and nutrient concentration in top layer due to leaf fall, and lower levels of N in deeper layers due to uptake by the trees).

Materials and Methods

Experimental design

In April 2013, *Salix* spp. (0.2 m cuttings) were planted on two quadrants of a 1-ha field (Figure 7.1). The clones were Tora (*Salix schwerinii*), Tordis (*S. schwerinii* x *S. viminalis*) and Klara ((*S. burjatica* x *S. viminalis*) x *S. burjatica*). These were planted according to the Swedish system (15,000 trees/ha, 75 cm between single rows, 150 cm between double rows, 60 cm between trees in each row). Between the rows, white clover (*Trifolium repens)* was sown as ground cover. On the remaining two quadrants, a grass-clover mixture was sown (commercial grass mixture consisting of 50% *Lolium perenne*, 20% *Poa pratensis*, 15% *Festuca rubra*, 15% *Phleum pratense* subsp. *Pretense*, supplemented with 1.5 kg of white clover per quadrant). An overview of the maintenance activities regarding the different types of vegetation can be found in Figure 7.2. In the winter of 2013/2014 the SRCW was harvested a first time, as this is sometimes done in practice to stimulate development of multiple shoots. The soil texture of the field was sandy loam, and the field was level. Average rainfall and temperature are displayed in Table 7.1.

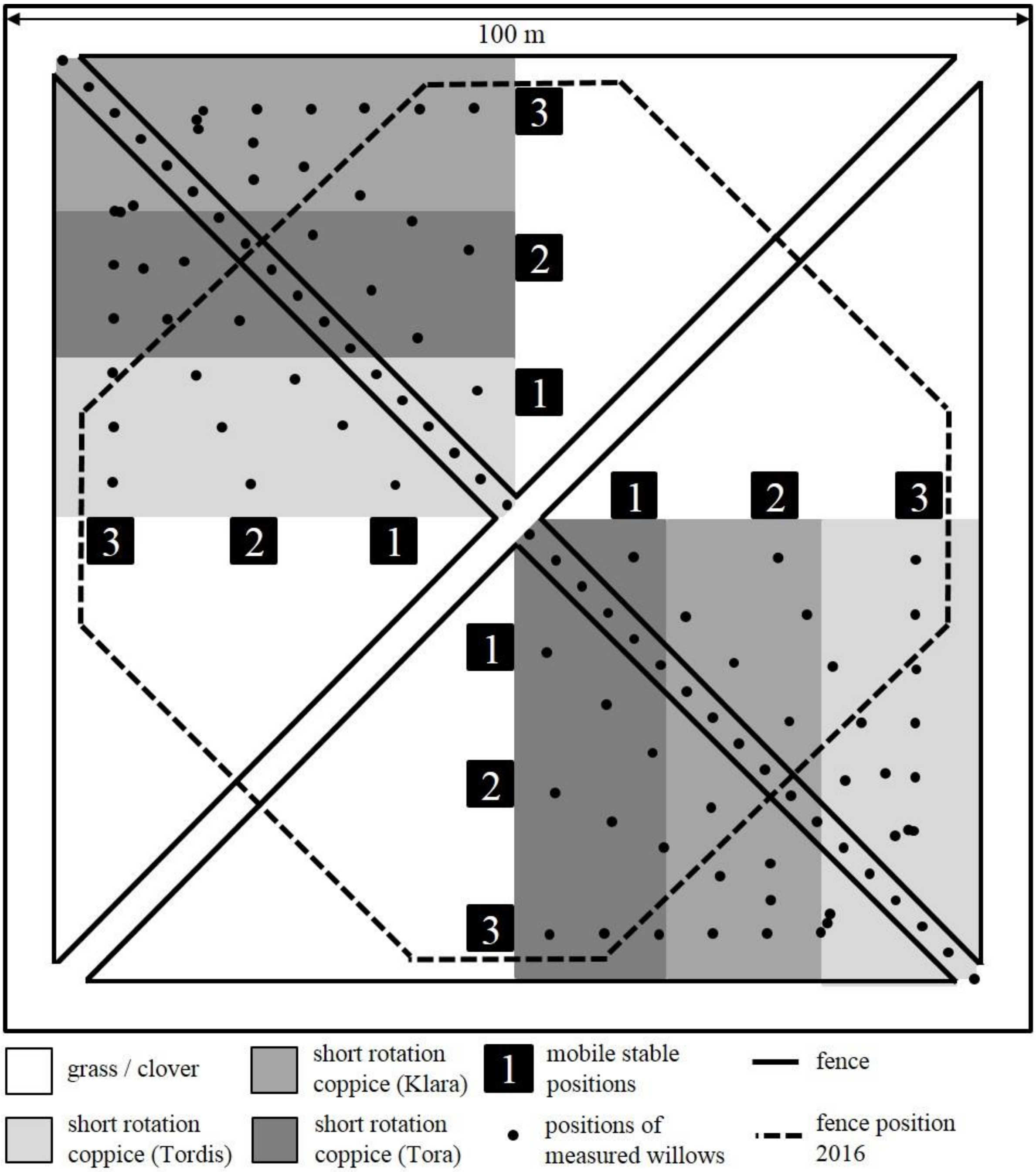


Fig. 7.1.a Map of the experimental field showing the different *Salix* spp. clones and the locations of the chicken houses and the individually monitored willows. Both grassland and both SRCW plots are ca. 42.5 x 42.5 m.

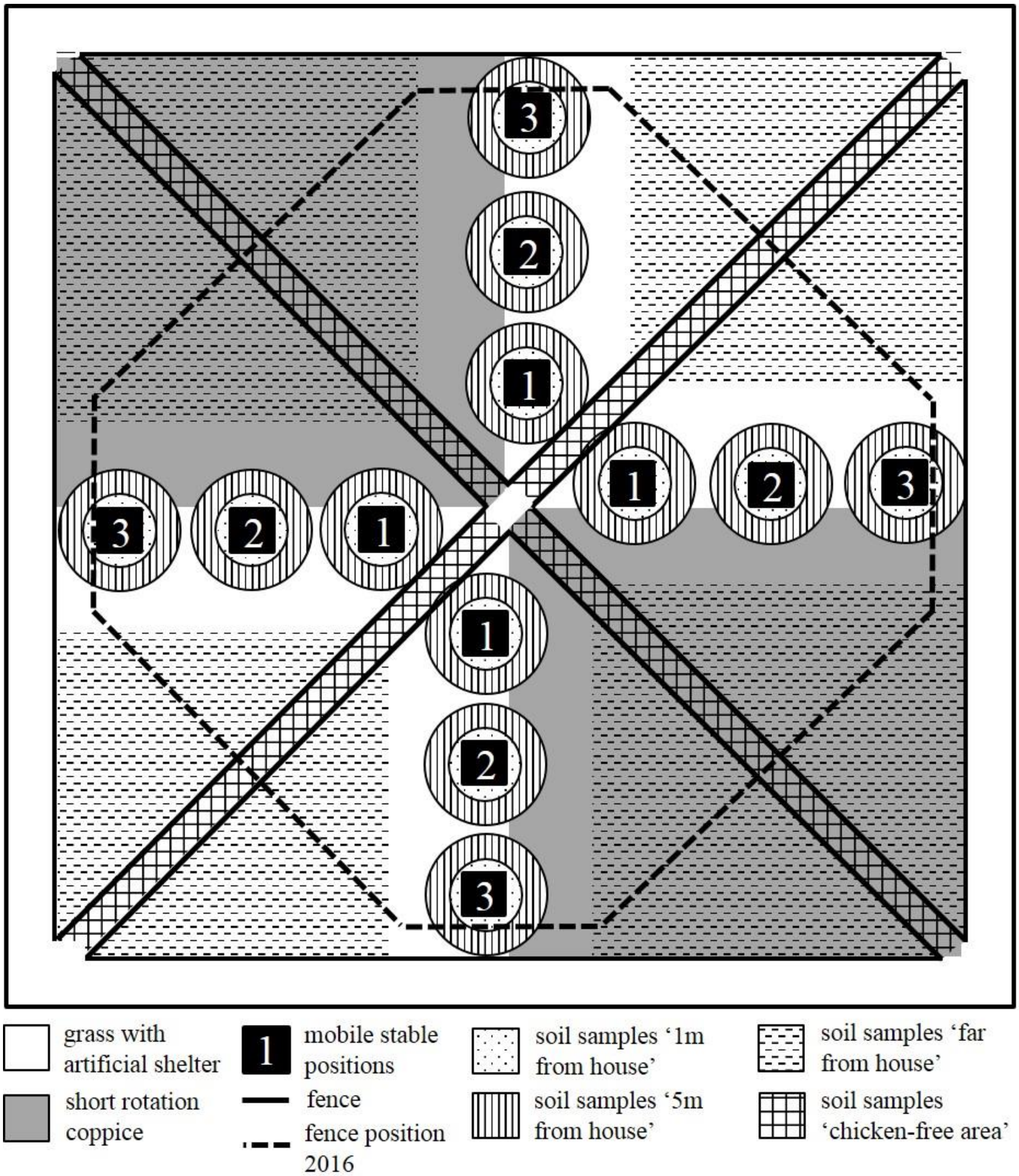


Fig. 7.1.b Map of the experimental field showing the different zones where soil samples were taken.

Table 7.1 Total rainfall and average, minimum and maximum temperature from 2013 to 2016, displayed for the entire year and the winter period (1 December of that year – 31 March of the next year) separately.

	2013		2014		2015		2016	
	Entire year	Winter	Entire year	Winter	Entire year	Winter	Entire year	Winter
Total rainfall (mm)	852	254	868	317	755	201	898	201
Average temperature (°C)	10.0	6.8	11.9	4.6	11.2	6.4	11.1	5.2
Min. temperature (°C)	-13.8	-2.2	-5.4	-5.4	-4.5	-6.0	-6.3	-6.7
Max. temperature (°C)	33.5	20.6	32.5	17.3	34.4	15.7	33.3	21.2

Using fences, the field was divided into four triangles each consisting of 50% SRCW and 50% grassland (Figure 7.1). A mobile chicken house was placed centrally on each of these fields. These housed 100 broiler chickens each, and were repositioned between the production rounds (Figure 7.1) in order to avoid point source pollution. Per production round, the chickens had access to the field for 6 – 7 weeks (depending on the exact slaughter date). In 2014, the houses were divided into two compartments so that birds of one compartment had access to grassland only, and birds of the other compartment had access to SRCW only (these were separated by a fence); in all other years they had access to the entire (triangular) field.

Between the four triangular fields that could be accessed by the chickens, there were two ‘chicken-free reference areas’, consisting of the diagonal strips crossing the field (Figure 7.1). This enabled quantification of the effect of presence of chickens on e.g. tree growth or soil parameters, by comparing parts of the field which could be accessed by the chickens with the ‘chicken-free reference areas’.

Measurements

Free-range use The first year (2013) was used for the SRCW to establish, and free-range use of the chickens was not monitored. In the next years the birds’ distribution was followed up systematically.

It was e.g. recorded how many birds were in SRCW and on grassland, and if these birds were within 2 m, between 2 and 5 m, or further than 5 m from the chicken house. More details on these observations were reported previously (Chapters 2-4 of this thesis).

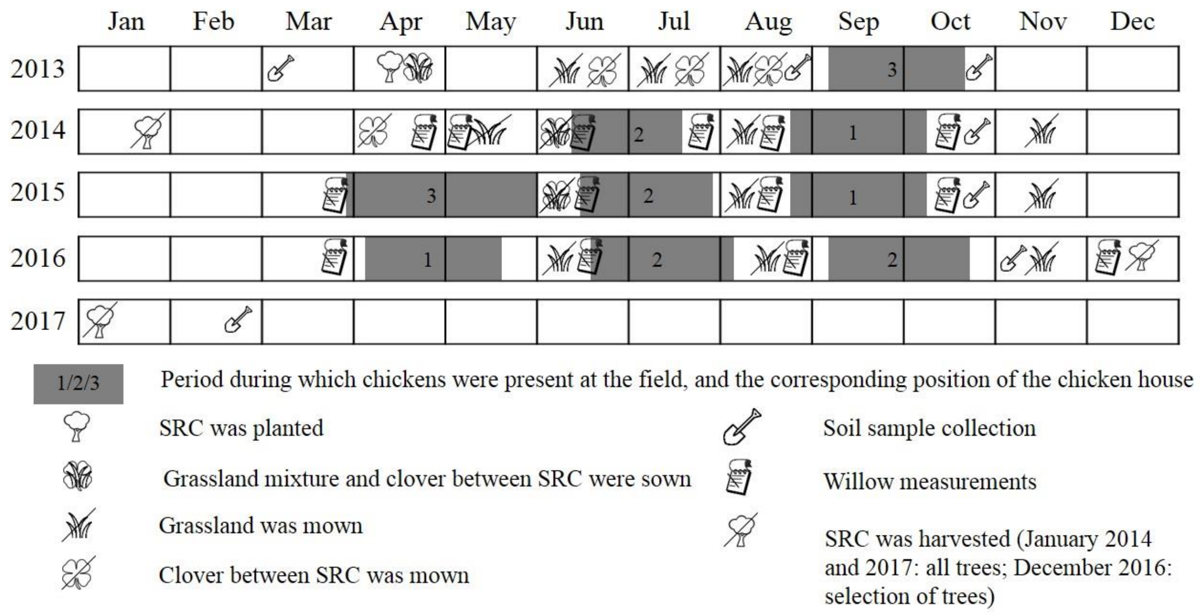


Figure 7.2 Overview of planting and maintenance activities on the field, timing of soil samples, willow measurements and chicken presence.

SRCW Number of shoots, diameter at the base of each shoot (with a digital calliper) and height of the tallest shoot (with a periscopic altimeter) were recorded regularly from 2013 to 2016 (Figure 7.2). A transect method was used to monitor tree growth. Three transect lines starting from the three house positions were marked, towards the outermost corner of the field (Figure 7.1). Along each line, six trees (inter-tree distance ca. 6 m) were labelled (n = 72) so that they could be followed up during two years. In addition, one tree per double row in the chicken-free areas was labelled and followed up (n = 36). Fifty-four of these labelled trees were harvested individually in December 2016 (first three labelled trees of every transect starting from the chicken house, and every other labelled tree in the chicken-free reference areas). From these harvested trees, fresh and dry weight were determined, as well as N and P content. In January 2017, the rest of the SRCW was harvested using a chain saw, after which the trees were shredded into wood chips. Both SRCW quadrants were divided into nine sub plots (for each clone per quadrant: one plot adjacent to each of the two chicken houses, and one within the chicken-free area), which were weighed individually. Wood moisture content was determined on a sub sample per sub plot to calculate the dry matter biomass. After the last harvest a sample of six rooting systems was excavated and basal area, fresh weight and rooting depths were assessed.

Leaf nutrient composition and nutrient input by leaf litter In order to quantify the amounts of C, N and P that falling leaves supplied to the soil, leaves were sampled and analysed. In 2014, just before completion of leaf fall at the end of October / beginning of November (depending on clone), leaf samples were taken. In both quadrants, leaves (10 per tree) from five trees per clone were collected from the top 30 cm of the trees. These were pooled per clone per quadrant and dried in a desiccator (7 days at 70 °C) and C, N and P contents were analysed. In addition, leaf fall was collected in baskets (40 x 45 cm wide, 15 cm deep) from the end of September until all leaves had fallen at the beginning of December 2014 and 2016. They were positioned in the chicken-free reference area in SRCW. These baskets were emptied regularly, leaves were dried and dry weight was recorded. These data were used to extrapolate the total amount of C, N and P returned to the soil by leaf fall.

To determine dry weight, fresh leaf samples were dried for 48h at 70°C. After drying, the plant samples were chopped to pass a sieve of 1 mm in a plant mill (Fritsch pulverisette 19). Foliar N concentration was determined according to ISO 16634-1 (Dumas method: Flash 4000, Thermo Scientific, USA), total C was measured by dry combustion at 1050°C using a Skalar Primacs SLC Total Organic Carbon (TOC) analyser. The total P concentration was determined for a subsample incinerated in a muffle oven (Nabertherm, Germany) for 4 h at 550°C. Subsequently, 10 mL H_2O and 10 mL HCl (6 N) was added to the ash, and evaporated. Then 10 mL HNO_3 (1.39 N) was added, and the mixture was boiled for 5 min. After filtration (Machary Nagel 640 m), the filtrate was mixed with ammonium molybdate and ammonium metavanadate reagent and diluted with H_2O. The P in the solution was measured in a spectrophotometer (430 nm) (Cary 60 UV-VIS, Agilent Technologies, USA).

Soil Soil samples were taken repeatedly, i.e. before the SRCW was planted, before the first chickens could access the field, every year in October/November and in February 2017 (exact dates and depths of sampling can be found in Table 7.2). For the first sampling moment the field was only divided into the four main quadrants (to assess the initial situation prior to the experiment), and for the second (prior to first chicken access) into two grassland quadrants and two SRCW quadrants divided per clone. Starting from October 2013, given the potential gradient in effect created by the heterogeneous presence of the chickens in the quadrants, each triangular field was divided into 14 distinct areas to take the soil samples: within 1 m from the chicken house (for three house positions) both on grassland and in SRCW, within 5 m from the chicken house (for three house positions) both on grassland and in SRCW, and far (>10 m) from the chicken house both on grassland and in SRCW (Figure 7.1). Additionally, each half of the diagonal strips making up the chicken-free reference areas (one on grass and one in SRCW) was sampled separately. In November 2016, which was the last autumn sampling of the experiment, most analyses were performed on the 0-10 cm layer, because most changes were expected to occur in this superficial layer, due to the relatively short time period of the study. TOC, however, was also analysed in the 0-30 cm layer because the rooting system of the SRCW may also have had an effect on this parameter.

Ammonium-lactate-extractable P, Mg, K, Fe, Ca and Mn (P-AL, Mg-AL, K-AL, Fe-AL, Ca-AL and Mn-AL) were assessed on sub samples oven-dried at 45°C, ground in a mortar and sieved over a 2 mm sieve, by extraction with ammonium lactate (AL, extraction ratio 1:20) in dark polyethylene bottles, shaken for 4 hours and the suspension was filtered in dark polyethylene bottles that were stored cool (4°C) until analysis. P-AL, Mg-AL, K-AL, Fe-AL, Ca-AL and Mn-AL were analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian Vista-Pro) with an axial torch. Organic carbon and Total N were measured on sub samples oven-dried at 70°C, ground in a mortar and sieved over a 250 µm sieve, by dry combustion at 1050°C using a Skalar Primacs SLC TOC analyser (ISO 10694) and at 950°C using a Thermo Flash 4000 N-analyser (ISO 13878), respectively.

P-$CaCl_2$ was measured with ICP–OES, after shaking (165 rpm) 10 g of fresh soil with 100 ml of a 0.01 M CaCl2 -solution for 2 h in dark polypropylene containers, and filtration on a Whatman N° 42 filter (NEN 5704,1996). Soil samples for the determination of the mineral N (NO_3^--N + NH_4^+-N) concentration were stored at -18°C until further analysis. Before analysis, the soil samples were thoroughly mixed in order to homogenize them. After, soil mineral N was determined in a 1M KCl extract according to ISO TS14256-1:2003 with a Skalar San++ continuous flow analyser.

In addition, a partial N balance was created, including assumptions for the amount of N deposited from the air (35 kg N/year; Stevens et al., 2004) and the amount of N removed by mowing the grassland. It was assumed that 6 x 10^3 kg DM / ha / yr was removed containing 3% N (Alburquerque et al., 2007; Gislum et al., 2004), which would equate to 180 kg N/ha) removed per year. For SRCW, 120 kg N/ha was removed by the harvest in 2014, and 300 kg N/ha by the harvest in 2017 (calculated from the average N content at harvest and the produced

Table 7.2 Overview of all collected and analysed soil samples. All samples were composite samples of at least five sub samples. NO_3-N = nitrate nitrogen; NH_4-N = ammonia nitrogen; TOC = total organic carbon; pH-KCl = pH; Amlact = Fe, K, Mg, Ca, Mn, Na and P extracted in ammonium lactate; N_{tot} = total nitrogen; $PCaCl_2$ = calcium chloride extractable P.

Date	Depth (cm)	Locations of samples	n	NO_3-N	NH_4-N	N_{tot}	TOC	pH-KCl	Am-lact	$PCaCl_2$
March 2013	0-30, 30-60, 60-90	1 per quadrant (2 grassland, 2 SRCW)	4	x	x	x[1]	x[1]	x[1]	x[1]	
August 2013	0-30, 30-60, 60-90	1 per grassland quadrant, and 1 per SRCW clone per quadrant	8	x	x	x[1]	x[1]	x[1]		
October 2013	0-30, 30-60, 60-90	chicken-free areas, far from house, 5 m from house (position 3)	20	x	x			x[1]		
October 2014	0-10	chicken-free areas, far from house, 5 m from house (position 1 and 2), 1 m from house (position 1)	36	x	x					x
	10-20, 20-30, 30-60, 60-90	chicken-free areas, far from house, 5 m from house (position 1 and 2), 1 m from house (position 1)	36	x	x					
October 2015	0-10	chicken-free areas, far from house, 5 m from house (position 1 and 3)	28							x
	0-30, 30-60, 60-90	chicken-free areas, far from house, 5 m from house (position 1 and 3)	28	x	x					
November 2016	0-10	chicken-free areas, far from house, 5 m from house (position 1 and 2), 1 m from house (position 2)	36			x	x	x	x	x
	0-30, 30-60, 60-90	chicken-free areas, far from house, 5 m from house (position 1 and 2), 1 m from house (position 2)	36	x	x		x[1]			
February 2017	0-30, 30-60, 60-90	far from house and 1 m from house (position 2)	16	x	x					

[1] *Only analysed in the 0-30 cm layer.*

amount of biomass), equating to 100 kg N/ha/year. Total N in the soil was measured in the 0-10 cm layer, and the difference between March 2013 and October 2016 was calculated.

Data analysis

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was evaluated at $\alpha < 0.05$, but trends ($P < 0.1$) were also taken into account. In case of post-hoc pairwise comparisons, the Tukey-Kramer adjustment for multiple comparisons was used at a total significance level of 0.05. Normality of the analysed data was assumed based on the graphical examination of the residuals (histogram and QQ plot). Non-significant interactions were removed from the models. Data are presented as LS means ± standard errors of the mean (s.e.m.) unless stated otherwise. For details on the analysis of free-range use see Stadig et al. (2016b, 2016c).

SRCW growth Data from December 2016 and the harvest in 2017 were used for analysis. Data from other measuring moments were used to monitor the growth over time, and were not analysed statistically. Diameter, height, and dry weight per tree (of the individually harvested trees and the average calculated from the total harvest) were analysed using linear mixed regression models with quadrant as random factor. For the measurements on individual trees (diameter, height and dry weight), the following fixed factors were included: distance from the house, clone and their interaction. Distance from the house was treated as a categorical variable, with the possible distances from the house (6, 12, 18, 24, 30 and 31-36 m) and 'in the chicken-free area' as levels (Model 1, Appendix A). For the dry weight per tree calculated from the total harvest, location of the tree (on the chicken range or in the chicken-free area) and clone were included as fixed factors (Model 2, Appendix A). One outlier for dry weight per tree of individually harvested trees was improbably high, perhaps because of a measuring error, and therefore excluded from the dataset. One measurement of the total harvest had an exceptionally low value for DM percentage, and was also excluded from the dataset.

Soil parameters Data of October 2016 were analysed using linear mixed regression models with quadrant as random factor. For N_{min} (NO_3-N + NH_4-N), vegetation (grass, SRCW), location (1 m from house, 5 m from house, >10 m from house, chicken-free reference area), depth (0-30, 30-60, 60-90 cm) and their interactions were included as fixed factors (Model 3, Appendix A). For NO_3-N and NH_4-N, total levels were used (sum of all three sampled layers, samples of 'old chicken house position' were excluded) and vegetation (grass, SRCW), location

(1 m from house, 5 m from house, >10 m from house, chicken-free area) and their interactions were included as fixed factors (Model 4, Appendix A). One outlier of NO_3-N and two of NH_4-N were removed from the dataset; these were data of 1 m from the chicken house with extremely high values which could be due to a high concentration of faeces at the sampling locations. Their removal did not affect the conclusions of the models, but did considerably improve the fit by improving normality of the residuals. In a separate model (with the measurements of the three layers separately) it was tested if depth interacted with vegetation or location (Model 5, Appendix A).

To assess the development of the N profiles over time, i.e. total NO_3-N and NH_4-N and N_{min} in the 0-90 cm profile (sum of all three layers) were modelled with date (October 2013, October 2014, October 2015, October 2016, February 2017), vegetation and location and their interactions as fixed factors, and quadrant and location ID as random factors to correct for repeated measures at the same location (Model 6, Appendix A). In addition, the differences in N_{min} between October 2016 and February 2017 were modelled to study possible leaching of N over the winter period. To this end, a model was constructed for each location*vegetation possibility, with date as fixed factor, and quadrant and location ID as random factors (Model 7, Appendix A). Similar to the data of October 2016, three outliers of NO_3-N and two of NH_4-N were removed from the dataset.

Data of October 2016 on P-$CaCl_2$, TOC, pH, K, Mg, Mn, Fe, P and total N in the top layer (0-10 cm) and TOC in the 0-30 cm layer were analysed using linear mixed regression models with vegetation, location and their interaction as fixed factors and quadrant as random factor (Model 8, Appendix A).

Leaf nutrient and wood composition Using linear mixed regression models, it was tested if N, P and C contents of the leaves were affected by clone. Quadrant was again included as a random factor (Model 9, Appendix A). The same models were used to test for differences between clones in amount of N and P removal from the field through harvest in 2017.

Results

Free-range use

In all years, free-range use was highest in SRCW compared to grassland. In 2014, when chickens had either access to SRCW or to grassland, free-range use was higher in the former groups (mean % of birds outside 42.8 vs. 35.1%; $P < 0.001$) and they ranged further from the house in SRCW (10.6% vs. 4.1% of the chickens outside that are more than 5 m from the house; $P = 0.002$). In 2015 and 2016, when all chickens could access both SRCW and grassland, they showed a clear preference for SRCW (average percentage of chickens outdoors in SRCW and AS, respectively, in 2015: 23.3% vs. 3.1%; $P < 0.001$ (interaction with distance from the house); 2016: 26.8% vs. 6.6%; $P < 0.001$ (interaction with week and distance from the house)). For more details regarding free-range use, see Chapters 2-4 of this thesis.

SRCW growth and development

In the winter of 2016-2017, mean tree diameter at the base of the shoot was 41.9 ± 13.6 mm (mean ± standard deviation). Mean tree height was 663 ± 125 cm. Mean dry weight biomass per tree of the individually harvested trees was 2.84 ± 1.71 kg. None of the measured variables was affected by clone, distance from the house or their interaction (Model 1; Table 7.3). Average dry weight of all trees, calculated by dividing total DM by the number of trees, was 3.03 ± 0.93 kg. This was not affected by clone or whether the trees were inside or outside the chicken range (Model 2; Table 7.3). However, numeric differences were considerable and degrees of freedom very low, and when removing quadrant as random effect from the model, clone (Klara 3.1 ± 0.2 kg, Tora 3.6 ± 0.2 kg, Tordis 3.0 ± 0.2 kg; $F_{2,12} = 3.60$; $P = 0.059$) did tend to have an effect. Total dry weight of all trees combined equalled a harvest of 16.0 x 10^3 kg DM / ha / year. Within the excavated sample, the rooting systems on average reached 20-40 cm deep, i.e., willows were rather shallow-rooted.

Soil parameters

Mineral N The initial levels of total mineral N (N_{min}; NO_3-N + NH_4-N) in the 0-90 cm layer ranged between 26.3 and 77.2 kg / ha in March 2013. N_{min} as determined in October 2016 was affected by a three-way interaction between vegetation type, location and depth ($F_{6,11} = 4.44$; $P = 0.016$; Model 3; Figure 7.3). Total NO_3-N (i.e. the sum of all layers within 0-90 cm; as determined in October 2016) was affected by location with higher levels close to the house ($F_{3,9} = 10.63$; $P = 0.003$) and vegetation with higher levels in SRCW ($F_{1,2} = 35.82$; $P = 0.027$), but not by an interaction between these factors (Model 4; Figure B.1 in Appendix B). When looking at the NO_3-N levels in the different soil layers (Model 5; Figure B.1 in Appendix B), there was an interaction between depth and location, with higher levels close to the house only in the 0-30 cm layer ($F_{6,17} = 5.01$; $P = 0.004$), but not between depth and vegetation.

Total NH_4-N (as determined in October 2016) tended to be affected by an interaction between location and vegetation, with higher levels in SRCW close to the house ($F_{3,5} = 4.34$; $P = 0.074$; Model 4; Figure B.2 in Appendix B). When looking at the NH_4-N levels in the different soil layers, there was an interaction between depth and location, with higher levels close to the house only in the 0-30 cm layer ($F_{6,17} = 3.27$; $P = 0.025$), but not between depth and vegetation (Model 5; Figure B.2 in Appendix B).

The N_{min} (NO_3-N and NH_4-N), profile concentrations from 2013 to 2017 were all affected by interactions between year and location, between year and vegetation and between vegetation and location (Model 6; Table 7.4). However, it was not possible to calculate modelled estimates for these effects or make pairwise comparisons, because not all combinations were present. Therefore, Figure 7.4 shows the means of the raw data of N_{min} to give an indication of N profile development over time per vegetation type.

When comparing N_{min} in the different soil layers between October 2016 and February 2017, there is a tendency for lower levels in February 2017 in SRCW at 1 m from the chicken houses (Model 7; Figure 7.5). N_{min} levels close to the house decreased in the 0-90 cm layer between these dates, indicating leaching to deeper layers within the 0-90 cm profile. Note that the degrees of freedom are low, indicating that statistical power was low, and that tendencies can therefore be considered as relevant in case of numerical important differences.

Figure 7.6 depicts the partial N balance that was created for SRCW and grassland. This balance shows that more N was removed from grassland by mowing than from the SRCW plots by the harvest, although the first was based on assumptions and the second on actual measurements. Over this 3.5-year period, the total-N level in the 0-10 cm layer of the soil has increased more in grassland than in SRCW plots.

Table 7.3 Diameter at the base, height, dry matter (DM) percentage and dry weight per tree (ls means ± s.e.m.) per clone and distance from the house or inside vs. outside the chicken range in the winter of 2016-2017 (nearly four years after planting). DF = degrees of freedom (numerator, denominator), F = F value, P = P value.

	Clone						Distance from house (m)									
	Klara	Tora	Tordis	DF	F	P	6	12	18	24	30	31-36	Chicken-free area	DF	F	P
Diameter (mm)	43.2 ± 2.8	38.2 ± 3.1	38.4 ± 3.0	2,2	1.65	0.377	43.3 ± 4.2	40.2 ± 4.2	34.6 ± 4.2	39.4 ± 4.2	39.5 ± 4.3	35.1 ± 4.3	47.4 ± 2.9	6,6	2.42	0.153
Height (cm)	682 ± 40	663 ± 36	635 ± 39	2,2	0.56	0.641	627 ± 43	675 ± 44	648 ± 44	NA	NA	NA	690 ± 38	3,3	0.69	0.616
Dry weight (kg) per tree (individual harvest)	3.1 ± 0.5	2.8 ± 0.5	2.5 ± 0.5	2,2	0.48	0.676	2.4 ± 0.6	2.6 ± 0.6	2.7 ± 0.6	NA	NA	NA	3.4 ± 0.5	3,3	1.11	0.466
	Klara	Tora	Tordis	DF	F	P		In free-range area				Chicken-free area		DF	F	P
Dry weight (kg) per tree (complete harvest)	3.1 ± 0.2	3.6 ± 0.2	2.9 ± 0.2	2,2	4.03	0.199		3.3 ± 0.1				3.1 ± 0.2		1,1	0.34	0.666

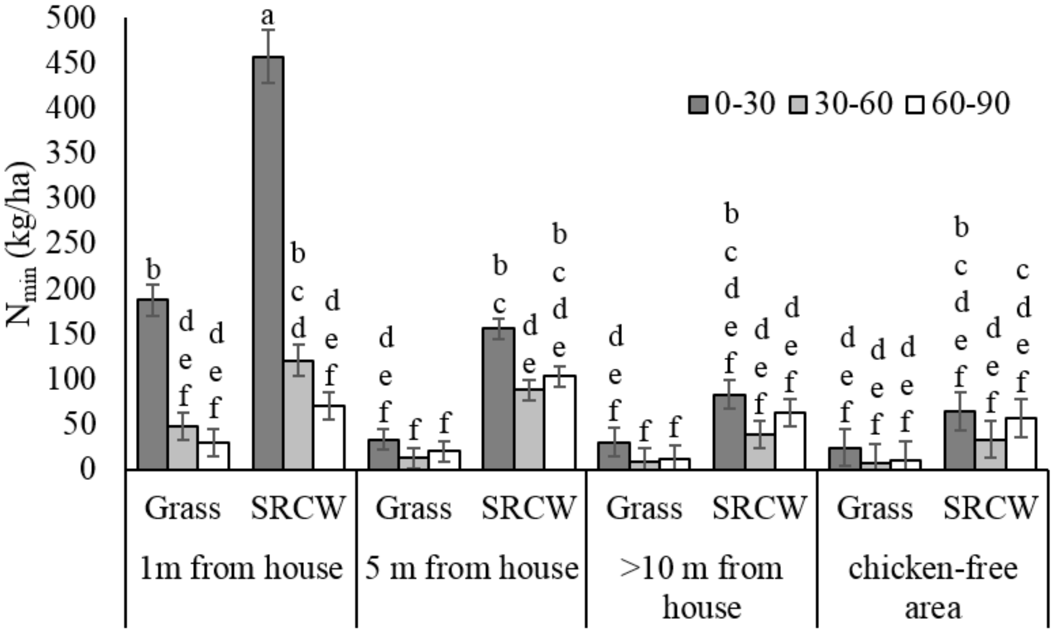


Figure 7.3 N_{min} (kg/ha) per vegetation type, location and depth. Bars without a common superscript differ significantly ($P < 0.05$).

Table 7.4 Effects of vegetation, location, year and their interactions on NO_3-N, NH_4-N and N_{min} between 2013 and 2016.

	NO_3-N			NH_4-N			N_{min}		
Effect	DF	F	P	DF	F	P	DF	F	P
Vegetation	1,32	34.89	<0.001	1,31	0.60	0.444	1,31	38.56	<0.001
Location	3,32	31.54	<0.001	3,31	28.83	<0.001	3,31	95.71	<0.001
Vegetation*location	3,32	5.19	0.005	3,31	6.33	0.002	3,31	17.63	<0.001
Year	4,32	22.15	<0.001	4,31	10.04	<0.001	4,31	22.16	<0.001
Year*location	7,32	10.72	<0.001	7,31	5.71	<0.001	7,31	10.85	<0.001
Year*vegetation	4,32	8.30	<0.001	4,31	2.76	0.045	4,31	10.91	<0.001

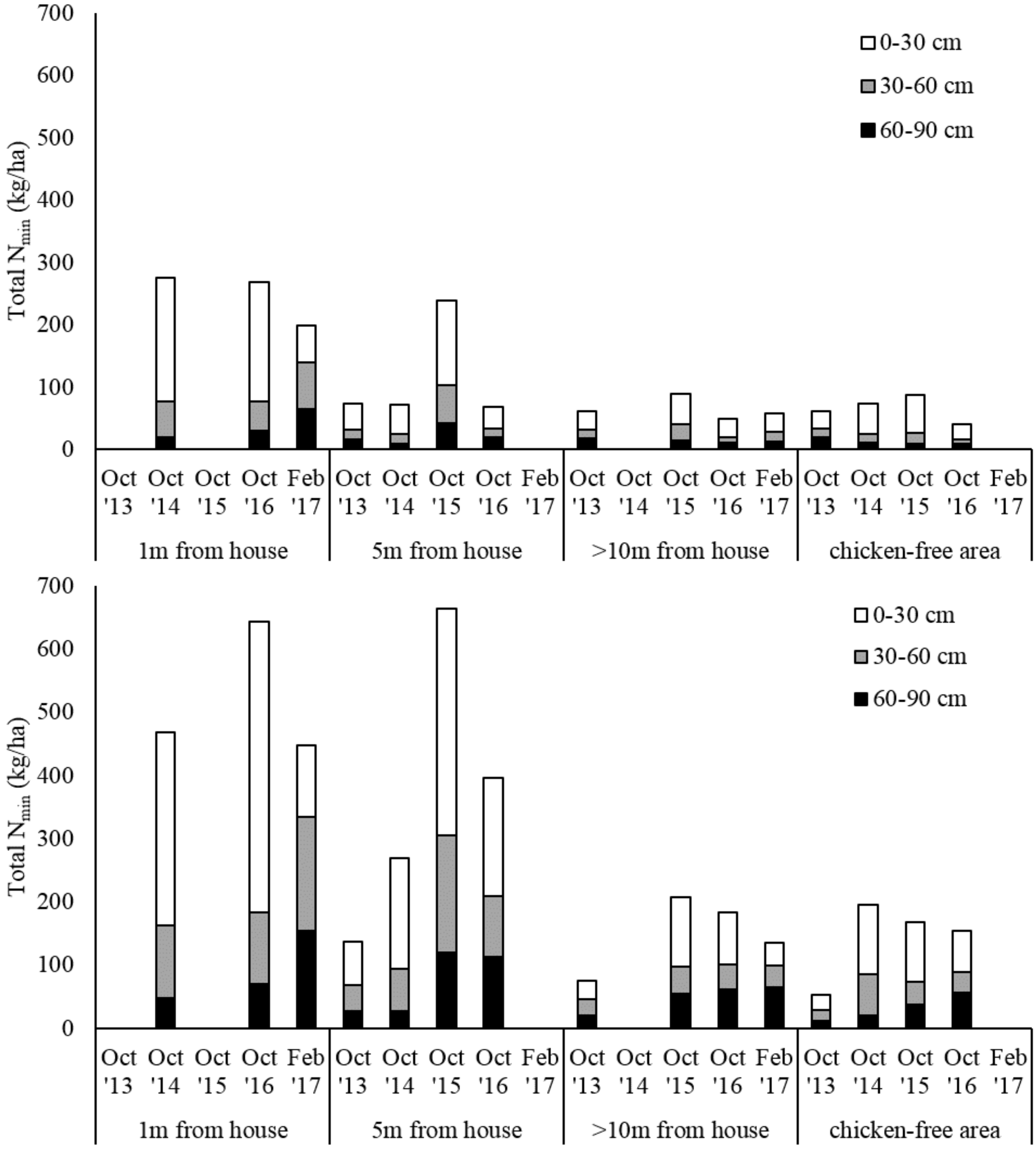


Figure 7.4 Evolution of N_{min} profile over time in grassland (above) and SRCW (below). Raw means are displayed because LS means could not be calculated. Dates without bars indicate N was not analysed for this specific date and location.

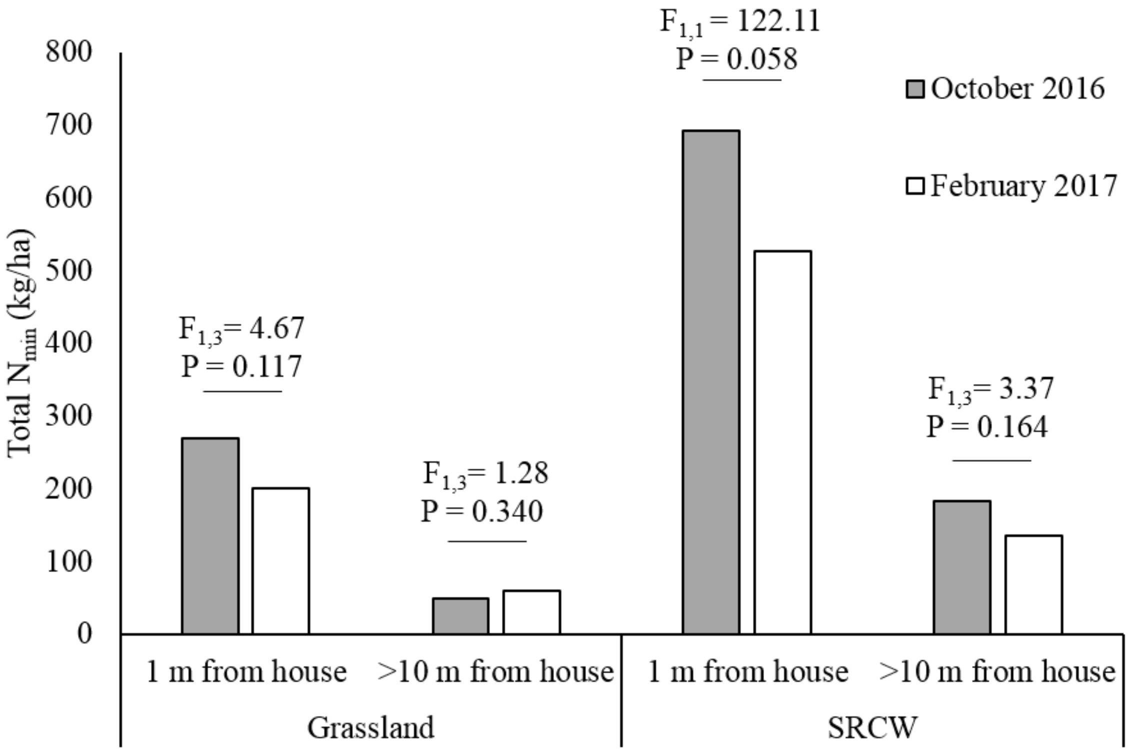


Figure 7.5 N_{min} in October 2016 and February 2017 (in 0-90 cm), per location and vegetation type. The F-test and P values indicate whether there were pairwise differences between October 2016 and February 2017 for this specific vegetation*location combination.

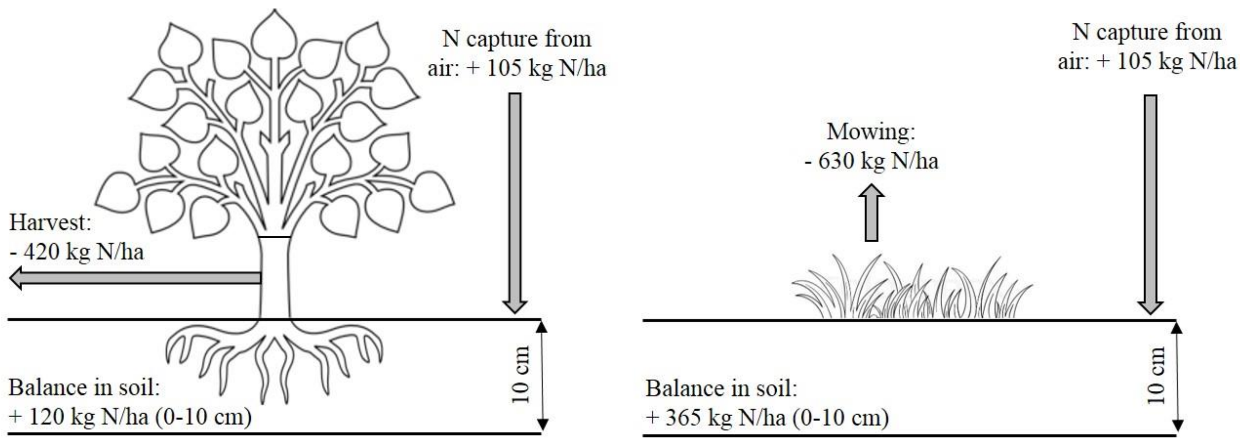


Figure 7.6 Partial nitrogen (N) balance over a 3.5-year period (March 2013 – October 2016) for short rotation coppice willows (SRCW; left) and grassland (right). Total-N levels were used for all calculations. N capture from the air and N removed by mowing were based on assumptions, N in the soil and N removed by SRCW harvest were based on measurements.

TOC, pH and other minerals The initial values of TOC, pH and other minerals are displayed in Table 7.5. None of the measures in the 0-10 cm layer (as determined in October 2016) were affected solely by vegetation type, but main effects of location or an interaction between location and vegetation did occur (Model 8; Table 7.5). TOC was higher at 1 m from the house than at >10 m (t_9 = 3.59; P = 0.006), and tended to be higher at 1 m than in the chicken-free reference area (t_9 = 2.81; P = 0.079). Soil pH tended to be affected by an interaction between vegetation and location, but no pairwise differences were found. P-$CaCl_2$ was not affected by vegetation (although it was higher in SRCW and there were only two degrees of freedom), but was higher at 1 m from the house than at other locations (all P < 0.037). Total N was higher at 1 m from the house than at >10 m or in the chicken-free reference area (both P < 0.021). K-AL in SRCW was higher at 1 m from the house than at other locations (all P < 0.003), but in grass no differences between locations were found. In SRCW, P-AL was higher at 1 and 5 m from the house (all P < 0.023). Mn-AL in SRCW was higher at 1 m from the house than at other locations (all P < 0.027), and at 10 m from the house, it was higher in grass than in SRCW (t_6 = 4.92; P = 0.003). Mg-AL was higher at 1 m from the house than at 5 m (t_9 = 3.26; P = 0.041) or >10 m (t_9 = 3.82; P = 0.018). Fe-AL was not affected by either vegetation or location.

Table 7.5 LS means (s.e.m.) of pH, total organic carbon (TOC), nitrogen (N), ammonium-lactate-extractable potassium (K-AL), magnesium (Mg-AL), manganese (Mn-AL), phosphorus (P-AL), iron (Fe-AL) and readily available phosphorus (P-$CaCl_2$) in the soil (0-10 cm) in October 2016. Test statistics (degrees of freedom, DF; F and P values) are given for the interaction between vegetation and location, or for their main effects (if no significant interaction was present).

	Default situation[1]		Grass				SRCW							
	Grass	SRCW	1m from house	5m from house	>10m from house	Chicken-free area	1m from house	5m from house	>10m from house	Chicken-free area		DF	F	P
pH	5.73	5.58	5.05 (0.15)	5.14 (0.15)	5.13 (0.15)	5.24 (0.20)	5.47 (0.15)	4.88 (0.15)	4.93 (0.15)	4.88 (0.20)		3,6	3.68	0.082
K-AL (mg/100g DM)	121	122	245^{b} (53)	81^{b} (53)	70^{b} (53)	115^{b} (67)	712^{a} (53)	285^{b} (53)	182^{b} (53)	157^{b} (67)		3,6	8.88	0.013
Mn-AL (mg/kg DM)	142	127	118^{ab} (7)	122^{ab} (7)	121^{ab} (7)	127^{ab} (10)	136^{a} (7)	83^{bc} (7)	71^{c} (7)	73^{bc} (10)		3,6	9.60	0.011
P-AL (mg/kg DM)	600	537	427^{ab} (32)	388^{ab} (32)	433^{ab} (32)	471^{ab} (35)	547^{a} (32)	430^{a} (32)	385^{b} (32)	385^{b} (35)		3,6	13.49	0.005
			Grass	SRCW	DF	F	P	1m from house	5m from house	>10m from house	Chicken-free area	DF	F	P
TOC (% DM; 0-10)	1.47	1.47	1.81 (0.05)	1.92 (0.05)	1,2	2.36	0.264	2.08^{a} (0.07)	1.93^{ab} (0.07)	1.73^{b} (0.07)	1.74^{ab} (0.10)	3,9	5.17	0.024
TOC (% DM; 0-30)	1.47	1.47	1.41 (0.03)	1.50 (0.03)	1,2	4.88	0.158	1.47 (0.04)	1.46 (0.04)	1.41 (0.04)	1.47 (0.05)	3,9	0.51	0.686
P-$CaCl_2$ (mg/kg DM)	NA	NA	5.0 (1.7)	10.3 (1.7)	1,2	4.70	0.162	14.0^{a} (1.8)	6.7^{b} (1.7)	4.7^{b} (2.2)	5.4^{b} (1.7)	3,9	7.10	0.010
Total N (% DM)	0.147	0.148	0.179 (0.005)	0.187 (0.005)	1,2	1.07	0.410	0.212^{a} (0.007)	0.188^{ab} (0.007)	0.164^{b} (0.007)	0.167^{b} (0.010)	3,9	8.99	0.005
Fe-AL (mg/kg DM)	1216	1099	953 (70)	769 (70)	1,2	3.51	0.202	804 (54)	845 (54)	884 (54)	911 (59)	3,9	2.61	0.116
Mg-AL (mg/kg DM)	162	158	131 (7)	143 (7)	1,2	1.51	0.345	163^{a} (8)	130^{b} (8)	124^{b} (8)	132^{ab} (11)	3,9	5.83	0.017
Ca-AL (mg/kg DM)	1468	1391	1175 (52)	1140 (52)	1,2	0.22	0.687	1126 (49)	1142 (49)	1165 (49)	1197 (61)	3,9	0.47	0.713

$^{a\text{-}c}$ Variables within rows (or within vegetation type or location, if no interaction was present) without a common superscript differ significantly ($P < 0.05$). [1] *NB: Average values of the two grass and SRCW quadrants, respectively, in the 0-30 cm layer from March 2013, i.e. before grass-clover was sown or SRCW was planted. Note that both the depth and the time of year differ from the other measurements (0-10 cm, October 2016).*

Leaf and wood nutrient composition and leaf fall

At the final leaf collection moment (i.e. just before leaf fall was completed) neither N ($F_{2,2}$ = 6.04; P = 0.142) nor C ($F_{2,2}$ = 0.33; P = 0.750) concentrations in the leaves differed between clones (Table 7.6). P concentrations were higher in Tordis than in Klara (t_2 = -6.32; P = 0.044) and Tora (t_2 = -9.74; P = 0.019; Table 7.6). On average, 922 and 1,569 kg of leaves / plot fell of the trees on each SRCW plot in 2014 and 2016, respectively (or: 2,552 and 4,344 kg / ha). This corresponds with 4.3 and 7.4 kg of P, 47.4 and 80.7 kg of N and 1,300 and 2,213 kg C / ha in 2014 and in 2016, respectively. N and P contents in the wood of harvested trees in December 2016 did not differ between clones (Table 7.6).

Table 7.6 Nitrogen (N), phosphorus (P) and carbon (C) content of the leaves (% of DM) per clone just prior to end of litterfall in 2014, and the amount of N and P exported from the field through harvest based on the individually harvested trees in December 2016.

	Klara	Tora	Tordis	DF	F	P
Leaves at end of leaf fall 2014						
P (%)	0.17 ± 0.01^{b}	0.14 ± 0.01^{b}	0.23 ± 0.01^{a}	2,2	48.86	0.020
N (%)	2.29 ± 0.1	1.75 ± 0.1	1.92 ± 0.1	2,2	6.04	0.142
C (%)	50.99 ± 0.10	51.05 ± 0.10	51.11 ± 0.10	2,2	0.33	0.750
Wood at harvest 2016						
P (kg/ha)	39 ± 2	38 ± 1	40 ± 2	2,2	0.61	0.620
N (kg/ha)	292 ± 12	319 ± 10	305 ± 11	2,2	1.57	0.389

$^{a\text{-}b}$ Means without a common superscript indicate a significant difference (P < 0.05) between the clones.

Discussion

Tree growth

Tree growth (assessed in terms of height, diameter at the base and dry weight biomass) was not affected by presence of chickens. This indicates that the extra nutrients supplied to the soil through chicken manure had no meaningful impact on biomass production, either by the relatively low amounts or slow release of nutrients supplied by manure or by a sufficiently high nutrient supply by the soil, indicating that nutrient availability was not the limiting factor for SRCW growth. Conversely, no negative effects of chicken presence were found. Such effects could arise from chickens foraging on the young leaves and plants, from exposing the roots of the trees by foraging behaviour, during which birds scratch away the soil with their feet, or from an excess of nutrients which might result in a higher occurrence of the willow watermark disease (De Vos et al., 2007). The time that chickens were present on the field was limited. A similar study with laying hens may yield different results, as these are kept for longer periods with range access (50-70 weeks). On the other hand, this may also imply more damage to the young trees. It is recommended that the trees are fenced off from the hens until they are 50 cm high (Boosten, 2015). In the present study, this height was reached in the first half of June in the first growing season, and in the first half of May in the second (data not shown).

Dry weight biomass of the trees was not significantly different between clones, which was possibly due to a low statistical power since the numerical differences between clones are considerable. If the dry weight biomass per tree is extrapolated to 10^3 kg DM / ha / year, this would give 15.5 for Klara, 18.0 for Tora, and 14.5 for Tordis. These yields are comparable with or higher than yields of these clones reported in previous studies (Albertsson et al., 2016; Finnan et al., 2016; Larsen et al., 2014; Stolarski et al., 2013).

Mineral N

The amount of N, P and other nutrients deposited on the field through faeces was not quantified. Excretion levels will depend on multiple factors such as diet, growth rate, and feed conversion. Fast-growing broiler chickens can excrete 0.25-0.43 kg N, 0.06-0.08 kg P and 0.19 kg K per bird-place per year (CBS, 2015; Kratz, 2002). However, slow-growing broilers are less efficient in their feed use so will excrete more nutrients (Kratz, 2002). Also, not all faeces will be dropped on the range; it has been estimated that up to 60% of free-range broilers' faeces are dropped outside (Kratz, 2002). In studies with laying hens, nutrient loads were calculated to be 673-2845 kg N, 123-736 kg P and 1074-1562 kg K / ha / year for the area within 20 m from the chicken house, depending on group size and season (Aarnink et al., 2006; Dekker et al., 2012). It was calculated that 20-45% of all faeces were dropped in these areas (Aarnink et al., 2006). If uptake by the SRCW is 80-100 kg N / ha / year (Aronsson and Bergström, 2001; Goodlass et al., 2007), the nutrients supplied by the chickens well exceeded this requirement, posing a risk for nutrient leaching in these areas. It has to be taken into account that these nutrient loads are based on studies with laying hens, which have outdoor access all year long, as opposed to broiler chickens, which have shorter production rounds and thus shorter periods with outdoor access (in Belgium, organic broilers get outdoor access for 5 weeks per production round, most farms have four production rounds per year, so chickens are outside during 20 weeks per year).

In October 2016, N_{min} and NO_3-N were higher in SRCW than in grassland, regardless of the distance from the chicken house. The same pattern was seen in October 2014 and 2015 (data not shown). This could be due to several reasons. First, N is returned to the soil through leaf fall in SRCW, as illustrated by the N levels in the leaves just prior to leaf fall, while the grassland was mown. This may mean that after a longer period, grassland may become depleted while SRCW maintains itself by returning N and other nutrients through leaf fall, although the clover that was present in the grass mixture will still also fixate N. On the other hand, N is also removed at SRCW harvest, although from the N balance it can be seen that this amount is smaller than the amount that is removed from grassland by mowing. Second, the chickens ranged more in SRCW than on grassland so more faeces will have been dropped in SRCW, although they were only present for a limited amount of time. As estimated above, a large part of N is returned by the faeces, but this N should mineralize before it is taken up by the vegetation or is measured as mineral N in the soil profile. Third, trees in proximity of chicken houses are able to capture up to 60% of emitted NH_3 from the air (Adrizal et al., 2008; Bealey et al., 2014; Patterson and Adrizal, 2005), and the fact that mobile houses but also other chicken houses were located in proximity of the trees in the current study implies their emissions may have contributed to higher N levels in SRCW. In the N balance, the N capture from air was assumed to be equal in both vegetation types, but nitrogen deposition in forests may be up to 75 kg N / ha / year compared to 35 kg N / ha / year in grassland (Dise and Wright, 1995; Stevens et al., 2004). Lastly, clover that was sown underneath the SRCW is able to fix atmospheric N (Boller and Nosberger, 1987; Ledgard, 1991). Clover was also present in the grassland, but was observed to strongly develop especially on SRCW plots in 2014 and 2015, possibly resulting in more N fixation in these plots.

It is difficult to separate the effects of vegetation and chicken density on the soil parameters, because chicken density was higher in SRCW. NO_3-N and N_{min} were always higher (although not always significantly different) in SRCW than in grassland, i.e. also in the chicken-free reference areas, showing there was a vegetation effect for this parameter. In both vegetation types NO_3-N and N_{min} decreased with increasing distance from the house, showing an effect of chicken density as well. The high levels of N_{min} in frequently used areas correspond with findings of Kratz et al. (2004). Jones et al. (2007) found no effect of chicken presence on NH_4, NO_3 or P levels in groundwater in an agroforestry setup, but it was unclear where exactly these samples were collected. In our study there was a numerical difference for NH_4-N between SRCW and grassland at 1 m from the house, but not at other locations. Taking into account there was 1-2 m between the chicken house and edge of the SRCW plantation (to enable opening and closing of pop holes), the difference at 1 m from the house is likely to be an effect of the chickens, although some leaves will also have fallen in this area. At locations farther from the house, no effects of chicken density or vegetation type were observed. Another reason for high N_{min} levels close to the houses was depletion of vegetation in these areas due to a high chicken density (informal observation), and therefore less N uptake by plants (Kratz et al., 2004).

In Belgium, the maximum allowed NO_3-N level at harvest (i.e., between October 1st and November 15th) is between 70 and 90 kg/ha, depending on soil texture; this was strongly exceeded at 1 and 5 m from the chicken house in October 2016. This concerned only a small area of the entire experimental field while the maximum allowed NO_3-N level should be assessed for the whole parcel, but these results nevertheless expose a risk for N leaching to groundwater. The data of February 2017 reveal a decrease of N_{min} in the 0-90 cm profile compared to October 2016, especially at 1 m from the chicken houses (decrease of 164 kg N_{min}/ha). When looking at the N_{min} in the different layers of the soil, a decrease is only observed at 1 m from the house and only at 0-30 cm; at 30-60 and 60-90 cm there was no decrease (in grassland) or an increase (in SRCW) over time. This strongly suggests that the N_{min} was leached from the top to the deeper layers, and was potentially leaching to the groundwater at 1 m from the house. At >10 m from the house, this decrease over time was not observed anymore (in grassland) or was much smaller (in SRCW), making it less likely that there was a risk of N leaching in this area. The area in between 1 and 10 m from the chicken house was not sampled and thus no conclusions can be drawn about this.

High levels of NO_3-N in groundwater during the establishment phase (the first year after planting) of SRCW have been observed in other studies, but concentrations decreased once the coppice was established (Goodlass et al., 2007; Mortensen et al., 1998; Nikièma et al., 2012). Nikièma et al. (2012) found 15 times higher NO_3-N levels in groundwater in newly established SRCW than in grassland, probably relating to soil disturbance. One year after establishment, NO_3-N levels were decreasing, and NO_3-N leaching was expected to reduce further as the trees grew taller leading to increased N demand and greater occupancy of the site (Nikièma et al., 2012). However, NO_3-N levels in the soil at 1 m from the chicken houses in SRCW in the present study at the end of a 3-year production cycle were comparable to those found by Nikièma et al. (2012) at the time of NO_3-N leaching. This indicates that there was a risk for

NO_3-N leaching in this part of our plots as well. Of course, other factors affecting leaching risk such as rainfall, soil structure and soil type also determine NO_3-N leaching (Aronsson and Bergström, 2001), and since we did not measure NO_3-N in groundwater we cannot draw conclusions on the amount of mineral N leached.

Our hypothesis was that in the deeper layers, the N_{min} could be taken up by the roots of SRCW. However, observations on the rooting systems showed these reached only 20-40 cm deep after four years of establishment, making uptake from deeper layers unlikely. This rooting depth is comparable with other studies (Crow and Houston, 2004; Rytter and Hansson, 1996; Souch et al., 2004). One study found rooting depths of 50-125 cm, which could be the reason for the reduction in N leaching after the establishment phase (Mortensen et al., 1998). In their study the soil type was loamy sand, which may have better facilitated root growth at greater depth.

P, K, TOC and pH

Chicken manure on average has an N:P ratio of 1.5 – 3.85 (Materechera and Mkhabela, 2002; Moore Jr. et al., 1995; Oonincx et al., 2015; Pederson et al., 2002; Xin et al., 2011). In comparison, in the present study the SRCW at harvest had an N:P ratio of 7.9. This discrepancy could lead to an accumulation of P in the soil when chicken manure is applied (Pederson et al., 2002). In the present study, P-AL and P-$CaCl_2$ as indicators of P load and readily available P concentrations, respectively (Vanden Nest et al., 2017), were indeed higher close to the chicken houses. In Belgium, P-AL levels are categorized into four levels depending on land type; the levels in grassland of this study would be in the second highest category, those of SRCW in the second highest or highest (depending on distance from the house; VLM, 2016). High levels of P-AL (123 to 375 mg/kg) in combination with high levels of P-$CaCl_2$ have been shown to indicate an increased risk for P leaching (Vanden Nest et al., 2017). These conditions were reached close to the chicken houses, as indicated by higher P-AL (547 mg/kg DM) for SRCW and higher levels of P-$CaCl_2$ for both vegetation types at 1m from the house (Table 4).

Levels of K were considerably higher at 1 m from the chicken houses than at other locations, and they were higher in SRCW than in grassland. This is most likely due to high levels of K in chicken faeces (Dikinya and Mufwanzala, 2010; Materechera and Mkhabela, 2002), and the higher chicken density at 1 m from the house in SRCW than on grassland. For grassland there is considerable export of K at harvest (estimated at 222 kg/ha based on 6 x 10^3 kg/ha/year being mowed, and the mean K content of grass (3.7 %; CVB, 2016)), while for SRCW the K is mostly recycled through litter fall and only partly removed with the wood.

TOC contents did not differ between grassland and SRCW in the 0-30 cm layer. Also, no difference was observed between concentrations in 2013 and 2016. It has been suggested that the potential for C sequestration is largest in soils with low C levels due to agricultural land use practices (Grogan and Matthews, 2002). Compared to other studies, C levels were quite low at the start of the current study (Dimitriou et al., 2012b; Jandl et al., 2012; Lockwell et al., 2012). The reason why SRCW did not result in increased TOC levels in the top soil (0-30 cm) is probably because of the short time period that SRCW was present (Lasch et al., 2010). It is expected that TOC will increase further over time, similar to other studies with SRCW (Schrama et al., 2016) or with yearly C application (D'Hose et al., 2016). In the 0-10 cm layer, TOC was lower at >10 m than at 1 m from the chicken house. This corresponds with studies applying chicken manure as fertiliser, resulting in an increase in TOC concentrations in the topsoil (0-10 cm), but not the subsoil (10-30 cm) layer. Chicken manure consists of 35-50% of C (Ranadheera et al., 2017; Warn, 2014), which explains the higher levels close to the chicken houses.

The pH in SRCW was numerically lower than in grassland except for at 1 m from the house, and in all locations lower in October 2016 than in the default situation. If the pH continues to decrease, this might pose a problem. Aluminium becomes soluble and, in this form, impairs root growth so that access to water and nutrients is restricted (Kochian et al., 2004). In addition, nutrients such as P may become unavailable (Kochian et al., 2004). Liming is the most common and economical method of ameliorating soil acidity; the required amount depends on soil pH profile, lime quality, soil type, farming system and rainfall (Gazey, 2017).

Possible remediating strategies

Although only a limited part of the field was subject to N and P leaching risk (parts close to the chicken house), regular rotation of the mobile houses between three locations alongside the SRCW-grassland boundary did not result in prevention of this point source of pollution. This is in accordance with findings of Kratz et al. (2004) who studied soil N and P concentrations of different broiler production systems, including systems with rotating range areas. Perhaps there was insufficient time before the house returned to the same location for vegetation to recover. It may thus be necessary to have more than three possible locations for the mobile houses in order to prevent N and P leaching to groundwater, or shorter rotation periods to prevent extensive damage to the vegetation. Another possible solution could be to provide concrete or another kind of impermeable layer around the chicken houses, or find more effective ways in achieving a better distribution of the birds over the range area. Harvest of the SRCW is not likely to alleviate the N load, since only part of the nutrients are removed from the field. If farmers have the option to choose between different plots for SRCW production, it would be best not to use plots with high soil levels of N_{min} because these could increase over time. Also, soils that are more compact or contain a high amount of clay could be less at risk of leaching.

Conclusions

SRCW was successful in promoting free-range use, and its growth was not positively nor negatively affected by the presence of chickens. However, the effects of chicken and SRCW presence on soil parameters, especially N_{min}, were not as expected, with higher levels in SRCW compared to grassland. The N uptake by the trees could improve over time, once the SRCW is more established. Alternatively, the results may indicate that the combination of chickens and SRCW is not ideal regarding the leaching risk for P and N_{min} in the soil close to the houses, although other studies (e.g. with laying hens, which are outdoor year-round, or with other soil types) are necessary to confirm this. In addition it is necessary to measure or model nutrient concentrations in groundwater to assess if leaching is taking place. If such studies would confirm increased N leaching, other strategies such as other rotation regimes or impermeable layers around the chicken house may be a solution to prevent N from leaching to groundwater.

Chapter 8

General discussion and conclusions

This thesis concerned combining slow-growing free-range broiler chickens with the production of SRCW. It focussed on promoting free-range use, on how free-range use affected other parameters such as leg health and meat quality, on how chickens and SRCW affected soil conditions, and on how to automatically monitor free-range use. The findings of this thesis regarding these topics will be discussed further, as well as practical implications of these findings and possibilities for future research. Finally, general conclusions will be formulated.

8.1 Promoting free-range use

In Chapter 1, several reasons for suboptimal range use and their remediating strategies were listed (Table 8.1). Figure 8.1 gives an overview of the factors found to influence free-range use in this thesis. The remediating strategies applied in this thesis will now be discussed in relation to the results presented in Chapters 2 until 7.

8.1.1 Adequate shelter

The first research objective of this thesis was **to assess the effect of different shelter types on free-range use**. The results of Chapters 2, 3 and 4 have demonstrated SRCW to be effective in attracting chickens to the range in comparison with AS. This could be related to SRCW providing better protection against adverse weather conditions and (aerial) predators, and to a more attractive environment for exploration.

Chapters 3 and 4 show that chickens strongly prefer SRCW over the AS used in this study (wooden A-frames). However, when only given access to AS, free-range use was lower than in groups with SRCW, but still high compared to other studies (Chapter 2). Over all study years, in total 27 – 43 % of the chickens were outside (AS or SRCW) at any given moment during the times they were given outdoor access. In comparison, other studies with broiler chickens found percentages between 5 and 13 % (Dawkins et al., 2003; Fanatico et al., 2016; Jones et al., 2007; Rodriguez-Aurrekoetxea et al., 2014). This indicates that also AS may be suitable shelters, although other factors may also have contributed to the high overall free-range use. The small group sizes (100 birds per group) may have played an important role in high free-range use as was demonstrated in earlier research for laying hens (Bestman and Wagenaar, 2003; Gilani et al., 2014; Whay et al., 2007). There are indications for increased behavioural synchronization in small flocks (Keeling et al., 2017), possibly leading to all birds going outside at the same time. Small groups are also housed in smaller houses, which may impede good climate control; such houses, made of plastic or wood, are often less well insulated than

Table 8.1 Reasons for low free-range use and possible remediating strategies (also shown in Chapter 1). Underlined strategies were studied in this thesis.

Reasons for low range use	Possible remediating strategies
Aversion to prevailing weather conditions	Adequate shelter on the range (SRCW?)
Fear of predators and/or new environment	Adequate shelter on the range (SRCW?)
	Gradual transition between indoor and outdoor
	Rearing method: reduce fearfulness
	Rearing method: early outdoor access
	Appropriate genetic strain
Low motivation to explore new environment	Rearing method: increase exploration motivation
	Provide suitable environment for exploration (SRCW?)
	Appropriate genetic strain
Social motivation / behavioural synchronization	Small group size
Physical inability (e.g. poor leg health)	Appropriate genetic strain (robust animals)
	Rearing method: stimulate activity
	Good litter quality
	No elevated pop holes
Motivation to stay in proximity of feed and water	Provide feed and water on the range
Time of day (low use at midday)	Provide outdoor access from early in the morning until the late afternoon or evening (depending on time of sunset)

larger, concrete buildings, and usually lack mechanic ventilation. In this study, the indoor and outdoor temperatures and RH were positively correlated, with the temperature tending to be higher indoors (Figure 8.2). This may have led to birds going outside to avoid heat stress indoors. The small group size is one of the limitations of this study; it is not known if all results are also valid for larger, commercial flocks. A Dutch project in which SRCW was planted in laying hens' free-range areas on commercial farms, so with large flocks, also demonstrated an increased use of the range with a better distribution of the birds over the range, although exact numbers were not reported (Boosten, 2015).

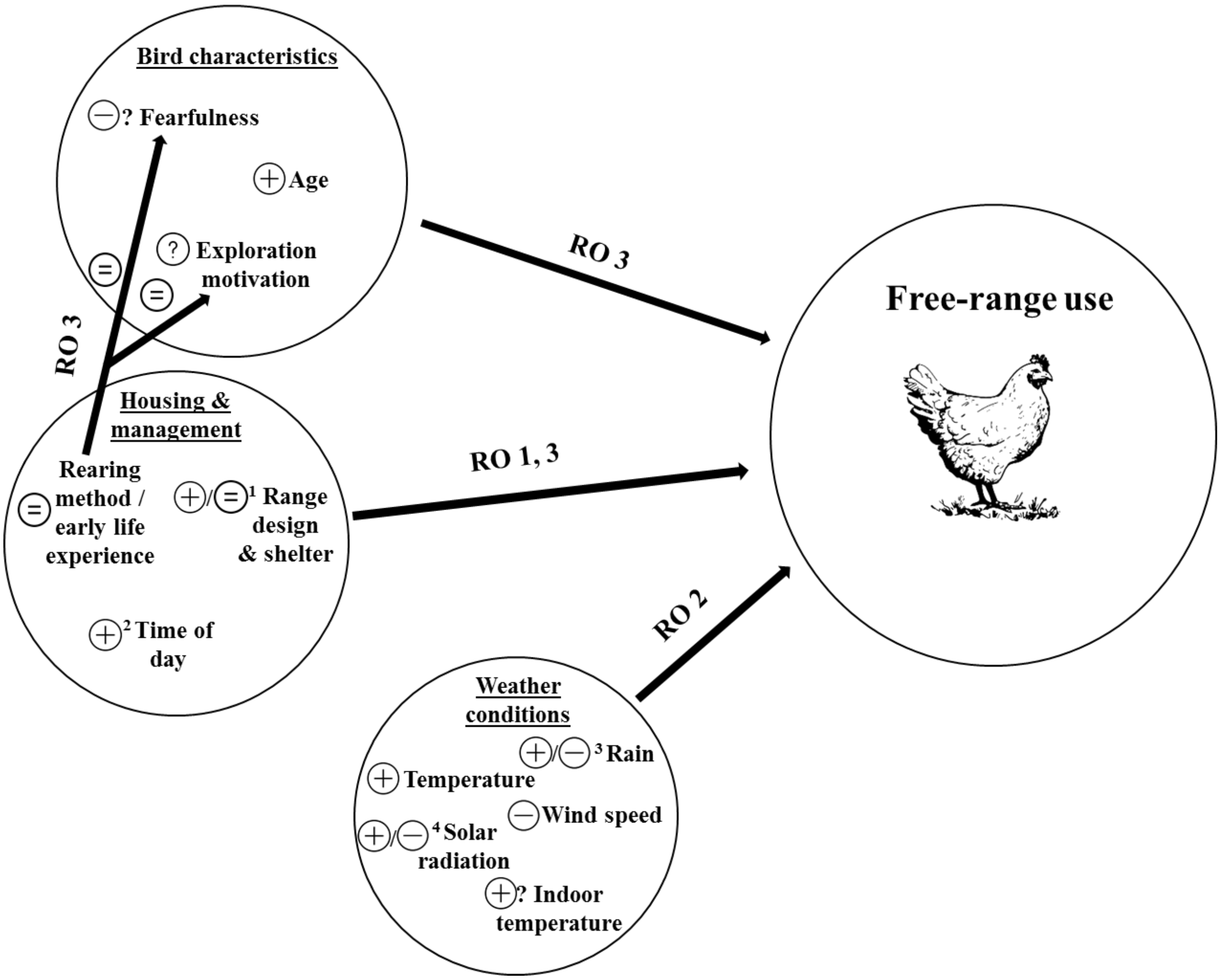


Figure 8.1 Schematic overview of how bird characteristics, housing and management factors and weather conditions affected free-range use. ⊕ indicates a positive effect of this variable on free-range use (or on fearfulness and exploration motivation in the case of rearing methods), ⊖ a negative effect, ⊜ no effect, ⓠ unknown, ⊕? and ⊖? a possible positive or negative effect. [1] Short rotation coppice willows (SRCW) increased free-range use compared to grass with artificial shelters (AS), but overhangs had no effect. [2] Birds ranged more shortly after the pop holes were opened and in the late afternoon. [3, 4] Effects depended on shelter type: rain increased free-range use in AS but decreased it in SRCW, for solar radiation the opposite was demonstrated. RO = research objective.

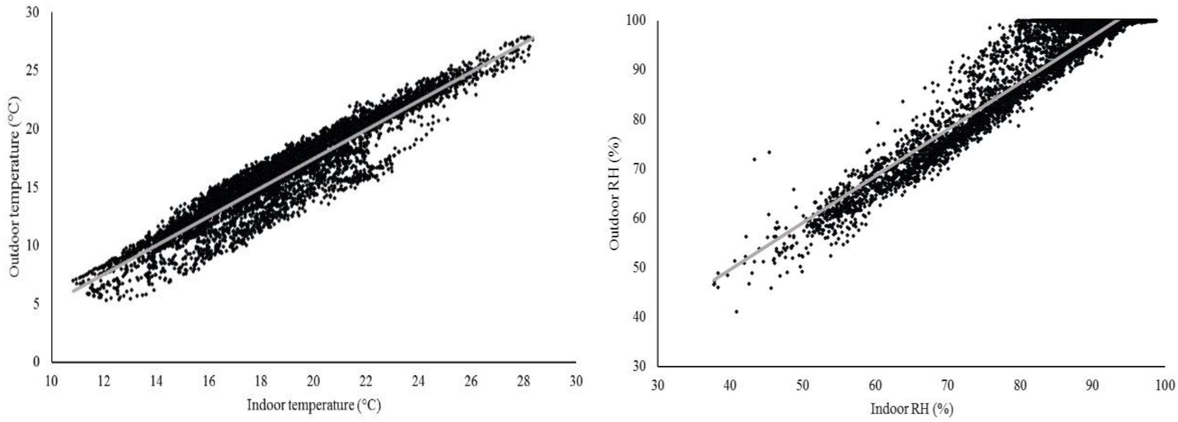


Figure 8.2 Relationship between indoor and outdoor temperature (°C) and relative humidity (RH; %; Stadig et al., unpublished data).

The second research objective was **to assess the effects of weather conditions on free-range use**, which was done in relation to the shelter types provided. Results of Chapters 2 and 3 indicate that decreasing temperatures (within a range of -1.9 to 33.6 °C), rainfall (range: 0 to 15 mm/15 min), increasing solar radiation (range: 0 to 1.01 kW/m^2) and increasing wind speed (range: 0 to 5.5 m/s) were related to fewer birds outside, indicating the aversion to these weather conditions. However, when birds were given the choice between AS and SRCW, increasing solar radiation was related to increasing numbers of birds in SRCW. This shows that high solar radiation is aversive to these chickens, but they do not necessarily go indoors if they can go to a more sheltered outdoor area instead. This is in agreement with findings of Fanatico et al. (2016) and Jones et al. (2007) and indicates the significance of the presence of suitable shelter on the range; suitable in this case meaning providing sufficient shade. Even though the A-frames may block more sunlight because they are solid, they do not cover the entire range, whereas SRCW provides a more continuous shelter against radiation. A lower solar radiation was correlated with a lower temperature in SRCW, as was shown in Chapters 2 and 3. It was shown in Chapter 3 that the number of birds in both shelter types decreased with increasing wind speed. However, the maximum wind speed observed in SRCW was lower than that in AS, indicating that SRCW provides better protection against wind. Although the number of birds in SRCW decreased when it rained, and those in AS increased, still more birds were observed in SRCW than in AS during rainfall (11.9% vs. 5.5%), indicating SRCW provided better protection against rainfall as well. The reason why the percentage of birds outside in AS increased when it rained is unclear. Perhaps more birds sought cover underneath the A-frames, this could be a topic for further research.

Results of Chapters 3 and 4, in which behaviours of outside birds were monitored, suggest that chickens feel safer in SRCW than in AS. More chickens (both relatively and absolutely) were observed to be sitting in SRCW than in AS, perhaps because they were more at ease. Alternatively, the chickens may have preferred the ground cover underneath SRCW for sitting compared to the grassland, or the microclimate may have been have been more favourable. Newberry and Shackleton (1997) showed that domestic fowl preferred areas with increasing amount of cover (up to 67%), and that they showed increased resting behaviour in these areas. These results could not be explained by thermoregulatory effects of the cover, which supports the hypothesis that the preference for SRCW arises at least partly from an increased sense of safety. In addition, more chickens (expressed in absolute numbers) were observed to be foraging in SRCW. Foraging has been shown to occur less in the presence of predator faecal odour, and is thus expected to occur more when birds feel safe (Zidar and Løvlie, 2012). In 2016 as compared to 2015, birds in SRCW preferred areas farther from the house. This could be related to a higher sense of safety due to a more developed vegetation (Figure 8.3), but could also be due to depletion of vegetation for foraging close to the houses.

The SRCW could have provided a more attractive environment for behaviours such as exploration, foraging or dust bathing. This is partly supported by a higher number of birds foraging in SRCW compared with AS. Chickens are motivated to explore feed and novel stimuli

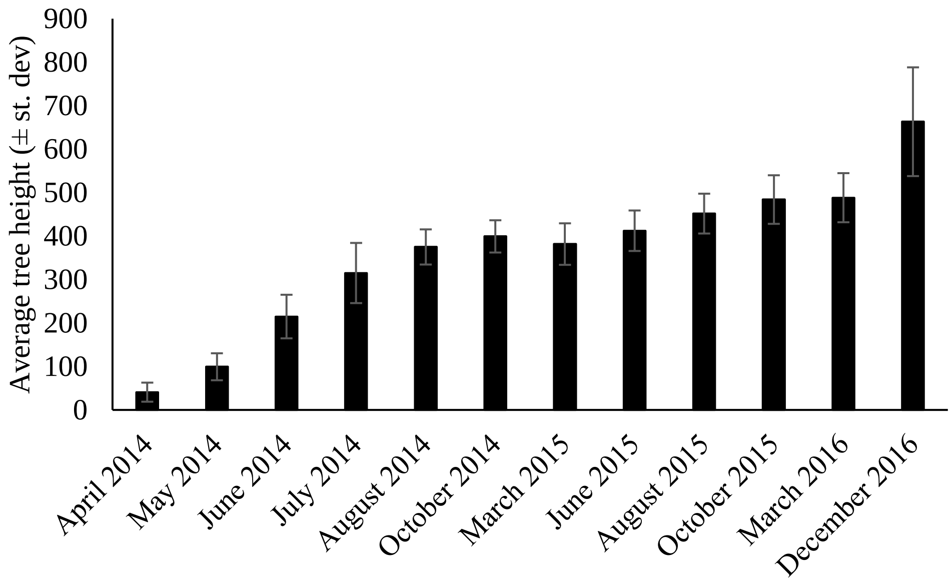


Figure 8.3 Average tree height between April 2014 and December 2016 (Stadig et al., unpublished data).

(Newberry, 1999). It is likely that an outdoor environment provides such stimuli, but plain grassland is probably less stimulating than areas with vegetation such as SRCW. The percentage of outside birds foraging was higher in 2016 than in 2015. This could be related to the increased attractiveness of SRCW due to canopy development, attracting more birds. Due to more canopy cover, there was less ground cover in 2016 than in 2015, which could have decreased foraging behaviour because chickens were mainly observed to be foraging on this vegetation, but the opposite was observed. This may indicate that amount of ground cover may not be key in motivating chickens to forage. Differences in dust bathing between shelter types were not observed, but this may be due to the sampling method (scan sampling with long intervals) which makes it less likely to observe this behaviour at all, since it only occurs ca. once every two days for each individual bird (Olsson and Keeling, 2005; Vestergaard, 1982).

The disadvantage of SRCW from a chicken point of view is that it is usually harvested every three years. An option could be to harvest one third of the plantation every year, so that sufficient sheltered range area remains at all times. Another advantage of such a harvest scheme is less need for storage of the wood chips, which is an economic benefit (see 8.3 for socio-economic aspects of SRCW). The question remains if other types of vegetation could elicit the same effects as SRCW. In order to answer this question, the characteristics of vegetation, such as density or quality of canopy cover, that is successful in promoting free-range use needs to be quantified.

8.1.2 Gradual transition

It was hypothesised that a more gradual transition from the house to the range would increase the number of birds going outside. However, the overhangs tested in this study did not succeed in promoting range use, as shown in Chapter 4. A recent study of Taylor et al. (2017a) showed that certain pop holes that were adjacent to shade cloth on the range were preferred by the birds, but others were not, suggesting there are other factors involved in pop hole preference. The lack of effect in the present study may be due to an unsuitable design of the overhangs (e.g. the sides were open while birds may prefer to walk along a more closed vertical structure (Dawkins et al., 2003; Pettersson et al., 2016b)), to the high overall range use making it difficult to further increase it, or to the fact that the transition may not have been a problem for these chickens because the difference between the inside of the mobile house and the outdoor environment was small. The latter may be different on larger commercial farms e.g. due to larger climatic and light intensity differences.

8.1.3 Rearing method

The third research objective was **to assess the effect of rearing strategy on fearfulness, behaviour and free-range use**. In Chapters 3 and 4, two early-life strategies aimed at increasing free-range use later in life were tested: environmental enrichment and dark brooders. It was hypothesised that these strategies would, among others, decrease fearfulness. The results from these studies were not conclusive. Environmental enrichment did not affect fearfulness, and had only small effects on behaviour and free-range use later in life. Dark brooders only tended to reduce the number of escape attempts in an OF test on day 22 or 23 of age, but did not affect free-range use or behaviour later in life.

The lack of effect of these strategies on fearfulness can be explained in several ways. Firstly, relationships between fearfulness and free-range use have been suggested in laying hens, but possibly fearfulness does not play such an important role in (these) broiler chickens. Although some studies found indications that broilers are indeed less fearful than laying hens (Keer-Keer et al., 1996; Saito et al., 2005), it has to be taken into account that in the present study slow-growing broilers were used, which differ from fast-growing broilers in many respects. They are for example more active and mobile, and may be more physically capable to show fear reactions such as fleeing than fast-growing birds. Broiler chickens have a relative short period of outdoor access compared to laying hens, so they have less time to habituate to this environment, and it is likely that fearfulness would especially be relevant during this first period of outdoor access because of the novelty of the environment. Secondly, raising all broiler chickens in small groups could have resulted in relatively low fear levels in all treatment groups (Bilčík et al., 1998). That would make demonstrating a difference between treatment groups more difficult. A relatively low level of fearfulness compared to other studies is however difficult to prove since measurements such as TI durations or behaviours in an OF test cannot be compared due to variations in e.g. genetics, housing and management. The lack of effect of the rearing strategies on free-range use may be related to the relatively high free-range use in all treatment groups, making it difficult to show any additional effects.

The possibility exists that the enrichment and the brooders were not sufficient in their type or design. Regarding the enrichment, the quantity of the mealworms may have been insufficient, and perhaps more different objects would have stimulated exploration more. The enrichment was observed to be used, especially the mealworms and the hay bales. A recent study showed that environmental enrichment early in life had some long-term effects, including effects on range use, in laying hens (Campbell et al., 2017). They showed that enrichment was associated with shorter but more frequent periods of range usage, so the link between environmental enrichment and free-range use may be more complicated than hypothesised. It was planned to observe dark brooder usage using video observations, but unfortunately technical errors were encountered and recordings could not be used. Anecdotal observations and state of the litter underneath the dark brooders after 25 days suggested dark brooders were, in fact, used.

It can be questioned if in studies where enrichment did affect fearfulness, this is due to a reduction in the fearfulness in general, or an increase in familiarity with the situation. In the study of Campbell et al. (2017) for example, enriched hens were provided with several physical enrichment objects, but also with coloured flashing lights and auditory playbacks including sounds of doors opening, moving vehicles, weather, voices, and machinery; these may have mimicked situations the hens would encounter outside and thus increased their familiarity with it. In a study by Reed et al. (1993) enriched hens that were also handled regularly showed less fear reactions during depopulation, which may again be due to familiarity (with humans). However, in other studies in which effects on fearfulness were found, there were no obvious links between the enrichment and the test situation. For example, Jones and Waddington (1992) provided brightly coloured, manipulable objects, which resulted in less freezing in a novel object test (perhaps due to familiarity), but also a shorter TI duration. Similar results were found in other species, e.g. rodents that were provided with objects such as tunnels and running wheels showed less fear for an electric shock (Barbelivien et al., 2006) and less anxiety-like behaviour in elevated-plus maze or free exploration tests (both models of anxiety; Chapillon et al., 1999). These studies suggest that enrichment has a long-term effect across situations, possibly by altering the HPA-axis reactivity or modifying the central nervous system, specifically the limbic system (Barbelivien et al., 2006; Chapillon et al., 1999). It would, however, be interesting to study if familiarity with structures and events that can be encountered outside would increase free-range use in chickens, even though this would not necessarily decrease their general level of fearfulness.

The period during which enrichment is provided is probably crucial in obtaining an effect throughout the birds' lives. Chicks go through different stages in which different forms of learning occur. Maternal imprinting occurs until 24 – 36 h after hatching, sexual imprinting follows at a later age but the exact period is poorly defined (10-12 weeks in male chicks; Appleby et al., 2004). In addition, they can learn to use certain resources if provided at a young enough age. Laying hens that had access to perches from 4 weeks of age onwards laid more of their eggs in nest boxes (due to perching experience) than hens provided with perches from 8 or more weeks of age onwards (Appleby et al., 1988). The exact timing of the sensitive period, if such a period truly exists in chickens, is unclear, and may depend on the type of stimulus and genetic strain of the birds.

Although fearfulness was not influenced by the rearing strategies tested in this thesis, the results in Chapter 2 suggest that it may indeed impact free-range use. A longer TI duration prior to the period with outdoor access was related to fewer chickens farther than 5 m from the house, both measured at group level. The percentage of birds outside was not related with TI duration. Other studies did find relationships between fearfulness and free-range use on an individual level, although in laying hens (Campbell et al., 2016a; Hartcher et al., 2016). It remains to be investigated whether such relationships also exist in broiler chickens.

It was also hypothesised that the two rearing strategies which aimed at increasing free-range use through reduced fearfulness (environmental enrichment and dark brooders) would increase motivation to explore. The effects of enrichment on behaviour were not as expected: no difference in foraging behaviour (reflecting explorative behaviour) was found, and enrichment was associated with a higher percentage of birds sitting or lying at later age. Dark brooders did not affect free-range use or behaviour later in life. Foraging behaviour was frequently observed; it was the most common behaviour in AS and the second in SRCW (after sitting or lying). Perhaps other factors such as genetic strain and a suitable environment had already sufficiently facilitated this behaviour. Exploration was also expected to be reflected in ranging behaviour, with more explorative birds ranging farther from the house. At >5 m from the house, foraging was the most observed behaviour indeed. A limitation of this study was that observations did not differentiate between areas once birds were farther than 5 m from the house. The dense vegetation made it difficult to observe the birds without disturbing them. Perhaps, the treatments did have an effect but only on the percentage of birds ranging farther than a certain distance from the house; this could be revealed by using an automated positioning system.

8.2 Effects of improved free-range use

Free-range use can affect multiple variables. Figure 8.4 gives an overview of the variables that were assessed in this study, and how these were related to free-range access and free-range use.

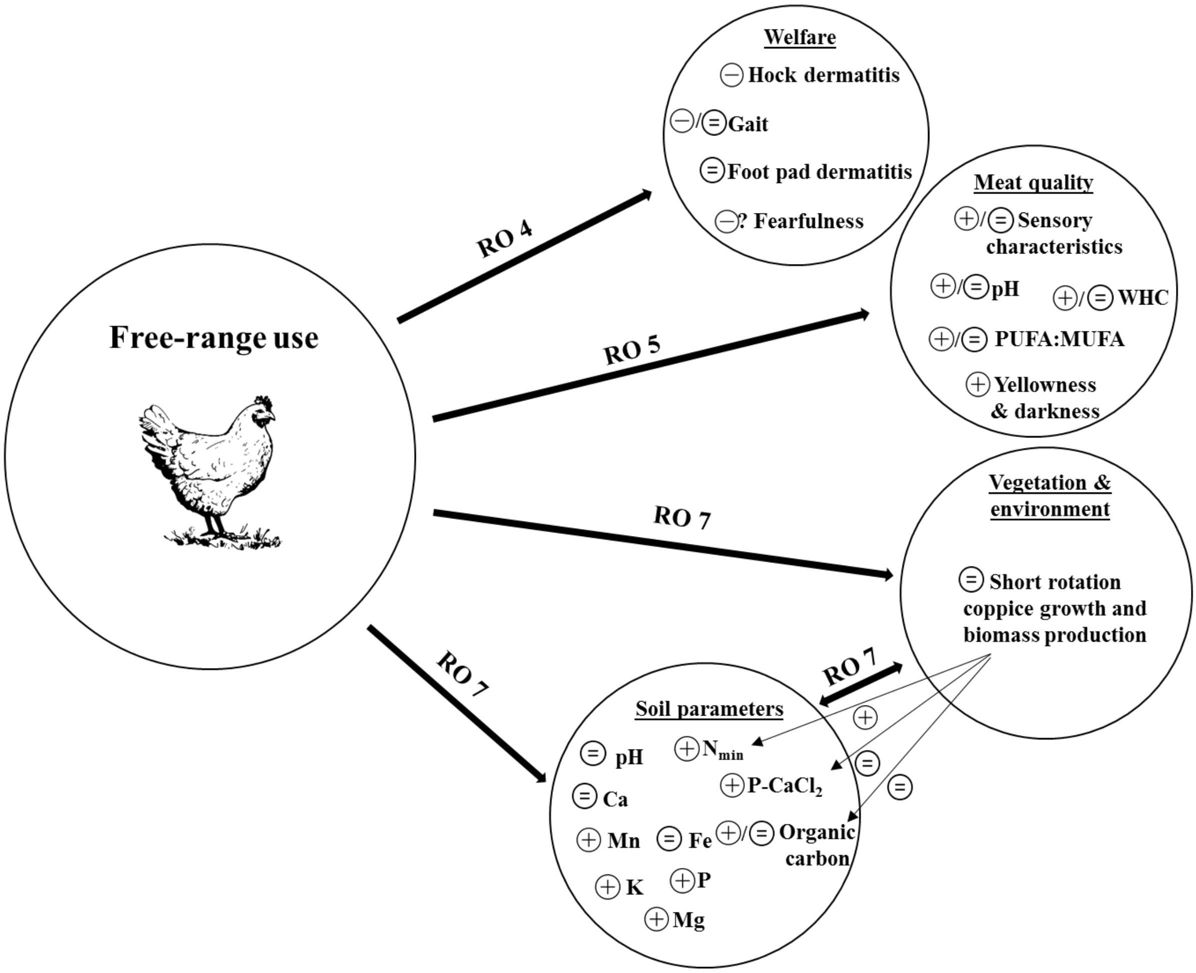


Figure 8.4 Schematic overview of the variables that were affected by free-range access and/or the level of free-range use, and how short rotation coppice willow presence affected soil parameters. ⊕ indicates a positive relationship, ⊖ a negative relationship, ⊜ no effect, ? means there were indications for a relationship but results were inconclusive. For more information on each of the variables, see their respective paragraph in section 8.2. WHC = water-holding capacity.

8.2.1 Leg health

The fourth research objective was **to assess the effects of free-range access and use on leg health**. In Chapter 2 it was shown that gait and hock dermatitis tended to be better in chickens kept with outdoor access, but no differences in FPD were observed. These findings can probably be attributed to the higher level of exercise in the birds with free-range access. The causality of the relationship between decreasing lameness and increasing range use in SRCW groups is unclear; it is not known if the birds ranged less due to lameness, or that they are lame (partially) because they did not range much. Recently, Casey-Trott et al. (2017) showed that increased opportunities to exercise (aviary compared to conventional cages) from week 1 to 16 positively affected bone growth characteristics in laying hen pullets. In the current study, limited relationships were found between range use and bone health.

Because free-range use was monitored at group level, it is possible that the birds that were selected for leg health assessment were not representative for their group in terms of free-range use. Ten birds of every group of 50 were tested; even though the range use of the whole group may have been high, it could hypothetically be that these ten birds were individuals that ranged the least. Therefore, individual monitoring of free-range use could be valuable, in order to more directly relate range use to leg health.

8.2.2 Fearfulness

The fourth research objective was also **to assess the effects of free-range access and use on fearfulness**. Results reported in Chapter 2 show that after the period with outdoor access, groups that used the outdoor area the most (those with SRCW) needed more inductions in the TI test than chickens kept without outdoor access, both measured at group level. This could be an indication for lower levels of fearfulness (Forkman et al., 2007). However, no correlation between TI duration and free-range use could be demonstrated. The exact relationship could be clarified using data from individual chickens. Other studies demonstrating relationships between individual free-range use and fearfulness have measured fearfulness during the period with free-range access (Campbell et al., 2016a; Hartcher et al., 2016). Therefore, it is unknown if animals that use the range more are intrinsically less fearful and therefore range more, or that they become less fearful because they range more, being exposed to more stimuli than indoor-preferring birds. Future research could focus on the development of fearfulness and free-range use over time.

8.2.3 Meat quality

The fifth research objective was **to assess the effects of free-range use on meat quality**. Chapter 5 showed that chickens with outdoor access had darker, yellower meat than those without, and that meat from chickens with access to SRCW was more tender and less fibrous than that of indoor birds or birds with access to AS, and more juicy than that of indoor birds. When looking at the conflicting results in literature on free-range chickens' meat quality, our results could be specific for the genetic strain, housing conditions, vegetation on the range, etc. used in our study. It is likely that there is a real effect of free-range access, given that this influences many factors such as diet and exercise. This could perhaps be quantified better if free-range use of individual birds could be monitored more closely. Nevertheless, in this study and other studies, differences between treatment groups are often small. Also, the consumer does not have the choice between birds that differed solely in if they had outdoor access; factors such as genetic strain, space allowance and diet will also be different between different products.

It can be questioned what is more important: the true differences between indoor and outdoor housed birds, or the perceived differences that the consumers experience when they know from which production system the meat originates. In a study in which a consumer panel tasted breast meat from conventional and organic origin with and without information on the origin, they were not able to differentiate between these if no information was provided (Napolitano et al., 2013). The expected liking (i.e. without tasting) was highest for the organic meat, and when they tasted the meat again, knowing the origin of the meat, their scores were higher than the original ones. However, not only the provision of this information but also the understanding of it by the consumer appears to play a role in consumer perception. Perceptions of aspects such as quality and overall liking by consumers that received a training about the labels prior to the tests differed between different product types (e.g. organic and not labelled), while perceptions by consumers without this knowledge did not differ (Samant and Seo, 2016). It could therefore be argued that as long as the consumer is educated about the label, the claims on the meat or consumers' expectations are more important than the actual differences in quality or taste. However, a pitfall of this strategy is that consumers have high expectations of the labelled product, which may not be met (Grunert et al., 2004; Napolitano et al., 2013). It is thus important that the quality of the meat lives up to the expectations of the consumer.

8.2.4 Vegetation and soil nutrient balance

The seventh objective of this study was **to assess interactions between presence of chickens, SRCW growth and soil nutrient balance**. In Chapter 8 it was shown that presence of chickens had neither positive nor negative effects on the growth of SRCW. It was hypothesised that the additional nutrient supply from the chicken faeces might have enhanced SRCW growth, but possibly the SRCW did not require any extra nutrients, or the supply was not sufficient to affect SRCW growth. Although no positive effects were demonstrated, it is important that no negative results were shown either, indicating that SRCW production in a chickens' free-range area is possible and not less productive than elsewhere. It needs to be noted though that in this study the chickens were only present during three periods of 6 weeks every year. It is known that when SRCW is planted on free-range laying hen farms, it needs to be fenced off during the first months in order to prevent damage to the young shoots (Stadig et al., submitted).

Nutrient loads of the soil were high close to the chicken houses, especially those of N_{min} and P. This has also been found in other studies, but it was hypothesised that SRCW would lead to lower levels of these nutrients through uptake and a better distribution of the chicken flock over the range. However, this was not the case, in fact, higher levels of most nutrients were found in SRCW compared to the grassland. This could be partly explained by a higher chicken density in SRCW, but the difference was also observed in those areas of the field that were kept chicken-free. The roots of the SRCW were probably not deep enough to take up nutrients from the deeper soil layers, and nutrients in SRCW were returned to the soil through litter fall while the grass, and thus the nutrients, was mowed and removed from the field. In SRCW, part of the nutrients is also removed at harvest, but as shown by the N balance in Chapter 7, the amounts are lower than in mowed grassland. The presence of clover probably also was of importance for levels of N in the soil, since clover can fix atmospheric N. Clover was sown underneath SRCW to decrease weed pressure, and was abundant on those plots in the first and, to a lesser extent, the second year after establishment; it disappeared once the canopy was dense enough. It was present on the grassland also mainly just after establishment, although not so abundant as on SRCW plots, but in the next years grass dominated. In the last winter of the study, nutrients seemed to migrate to deeper soil layers, but not all nutrients disappeared from the soil, and these could possibly be taken up again by the vegetation in the next growing season. In order to make conclusions about actual leaching to the groundwater, this will have to be measured directly. Alternatively, the risk for leaching to the groundwater could be modelled based on the parcel-specific data (Hansen et al., 2000).

Besides effects on growth and N and P load, other interactions between the chickens, vegetation and soil may be present. It was expected that SRCW would increase C sequestration as compared to grassland. The results did not confirm this, although it is expected that in time a difference will be observed as this is a long-term process. In addition, the present study initially attempted to quantify the effects of chickens on the presence of willow beetles, which are plague insects capable of doing substantial damage to SRCW plantations (Peacock et al., 1999). This proved not possible due to the small size of the experimental field combined with the mobility of the beetles; the areas where the chickens could range were too close to the neighbouring chicken-free zones so that the beetles could move freely between them. This would be an interesting pathway for future research. This could for example be done with smaller groups of chickens and smaller free-range areas than in the studies in this thesis, so they can be physically separated by nets. Then, the comparison can be made between areas with and without chickens, without the beetles being able to move between these areas. This may be difficult because small openings in the nets could already let insects through. Alternatively, beetle presence and damage to the trees could be determined at different locations that are sufficiently far apart from each other, so there can be no ‘cross contamination’ of beetles. However, this means that environmental factors can also play a role, so it will need more replications of plots with and without chickens.

Overall, the environmental feasibility of combining SRCW with free-range broiler chickens is uncertain. High levels of N and P in the soil, indicating a risk at leaching to groundwater, are not very sustainable. One option would be to limit the period of time that a plot can be used as free-range area; however in practice this is not feasible because farmers often are limited to the plot of land adjacent to the poultry house. Also, if SRCW is planted, this is usually done for a period of 21 years and not shorter. A possible solution could be to further study strategies to achieve a more even distribution of birds over the range, instead of a high concentration close to the houses. Alternatively, if mobile houses are used these may require more frequent repositioning. Compared to conventional broilers, free-range or organic broilers require more space, and more feed to produce the same amount of meat (so also more land to produce feed), so the input required is higher. However, organic production is associated with a lower eutrophication and acidification potential, less risk of antimicrobial resistance, and a lower impact on biodiversity (Van Wagenberg et al., 2017).

In addition, it appears difficult to close the nutrient loop (feed → chicken → faeces/manure → feed) due to the faeces ending up in the soil. A possible solution for that could be using an impermeable layer or wood chips in areas with high chicken density, that could later be removed and composted. An additional ‘problem’ is that chicken feed consists for a big part of soy, which is not yet produced in large quantities locally, so the manure is not likely to be re-used in the production of chicken feed. This could lead to regional nutrient excesses. The soy is needed because it is a suitable protein source, which is important because it is not allowed to add synthetic amino acids to organic poultry feed. The latter causes higher N excretion because the amino acid balance is not always optimal. It is also not allowed to add phytase to organic poultry feed, causing a higher P excretion (Waldroup et al., 2000). Poultry manure is usually burnt as waste because it is not attractive as fertiliser due to the relatively high P content; pig or cattle manure is preferred. In Belgium and the Netherlands, phosphorus levels are usually already high. An option that could be investigated further is the provision of vegetation in free-range areas with high concentrations of available and suitable nutrients for the chickens, to partially replace the need for soy.

8.3 Socio-economic feasibility of SRCW in free-range areas

From the preceding paragraphs it can be concluded (1) that SRCW had positive effects on free-range use, (2) that this increased free-range use can result in improved meat quality and chicken welfare, but also (3) that high levels of N_{min} and P in the soil under SRCW may be a point of attention. However, other aspects also need to be taken into account to judge the suitability of SRCW, such as farmers' motivation for and concerns about planting this vegetation, because they are the ones who decide to implement it or not.

Interviews with 17 free-range laying hen farmers and one free-range broiler farmer showed that the majority acknowledged the potential of SRCW to improve free-range use and animal welfare (Stadig et al., submitted). They were aware that chickens descended from the jungle fowl, and hence that woody vegetation is an important component of their natural habitat. However, they also thought that the dense vegetation would increase predation by foxes, that it would keep the chickens from coming into the chicken house at night and from laying eggs in the nest boxes, and that it would be more difficult to check for dead chickens on the range. Nevertheless, some of the interviewed farmers already had experience with SRCW and they did not report an increased predation by foxes or a decrease in production from chickens laying their eggs outside. Another concern of many farmers was the labour required to establish and maintain an SRCW plantation. This was confirmed by the farmers with SRCW, although most of the labour was restricted to the year of planting; good weed control was essential in this period. The farmers with SRCW had only planted this less than three years ago and did not yet harvest it, so they could not predict the labour required after the first harvest. Studies indicate that less weed control is needed after establishment of the plantation, but that tall weeds can still result in reduced biomass production (Larsen et al., 2014; Sage, 1999).

An economic analysis of implementing SRCW was performed for several scenarios, representing average-sized broiler chicken and laying hen farms (for Flanders), at which the wood chips were either used for on-farm heat production or sold (Stadig et al., submitted). This showed that in most cases, the net present value (NPV) of the investment was positive after a period of 23 years. The NPV is the current value of all future cash flows resulting from an investment, taking into account the initial investment as well. It is a method that takes a discount rate into account for future costs and revenues. Although the NPVs were positive in most cases, they were generally also low, ranging from € 1 681 to € 2 154 in the default scenarios (over a 23-year period), which were the scenarios where the input variables were set at the currently most likely or mean value. In a sensitivity analysis, several of these input variables were varied between their lowest and highest realistic values. A higher wood chip price, a higher gas or oil price and a price premium for the eggs or meat were identified as factors that could result in a substantially higher NPV. Scale effects were not taken into account because it was assumed that these would not result in substantial benefits taking into account the size of the average free-range area. Such effects may play a role if local poultry farmers would cooperate, or if an external company (e.g. a bioenergy company) would be responsible for establishing, maintaining and harvesting the SRCW. These options may be interesting both from a practical and an economic point of view. A risk analysis, in which all input variables were varied simultaneously in 10 000 iterations, showed that the NPV was positive in 79.8 to 93.0% of the cases, with the highest percentages in the scenarios in which the wood chips were sold. In conclusion, SRCW in chickens' free-range areas can be economically profitable; although margins are currently small these depend on many variables, providing several options for enlarging these margins.

8.4 Applications of Ultra-Wideband tracking

The sixth objective of this thesis was **to develop an automated system able to monitor free-range use of individual birds**. Chapter 6 describes the accuracy and signal reception of the UWB system that was developed. It shows great potential, with the mean accuracy below the requested 50 cm in 60 to 90% of the cases, depending on the subfield. However, it has to be acknowledged that in some cases large errors occurred, as well as poor signal reception. These problems occurred mainly on fields 2 and 3, and could be due to the setup of the anchors, which was not completely symmetrical, possibly disadvantaging these fields. It is expected that these problems can be solved by placing more anchors on the field, but this remains to be tested. The percentage of positions that could be registered was lower in SRCW than on grassland, which could be due to problems with the functioning of two anchors adjacent to the SRCW plots. It is not yet exactly known how the system will function when the tags are placed on moving chickens. Tests were performed where the tag was either fixed on a wooden stick or on a chicken (in a cardboard box), which were then moved along a straight line. Visual assessment of these data reveal good alignment of the registered positions along the travelled trajectory, with some outliers, but these were comparable to those from the stationary tests.

The aim of this thesis was to get insight into how to promote free-range use, so that strategies to improve this could be used in practice. The APS could play a role in this. There are indications that fearfulness has a genetic component (Bolhuis et al., 2009; Campler et al., 2009; de Haas et al., 2010; Gallup, 1974; Jones, 1977; Jones et al., 1995; Jones and Faure, 1981; Jones and Mills, 1983; Korte et al., 1997; Nordquist et al., 2011; Uitdehaag et al., 2008), that it is related to free-range use (Campbell et al., 2016a; Hartcher et al., 2016; Mahboub et al., 2004) and that free-range use itself can also be influenced by genetic selection (Icken et al., 2008). So, if free-range use of broiler breeders would be monitored at an individual level, those that range most could be selected for developing genetic lines that are most suitable for production systems with outdoor access. Unfortunately, not many broiler breeders are kept with outdoor access, even on organic farms. So, alternatively, the APS could be used to assess which other measure, e.g. a behavioural test, could be used as a proxy of free-range use. For example, free-range use may be correlated to activity in an OF test, or proximity to touch a novel object.

Since it is difficult and time-consuming to consistently and reliably measure behaviour to evaluate large numbers of animals needed for a breeding programme (D’Eath et al., 2010), an automated system could assist in this. Some may perceive such breeding programmes as artificially creating docile animals, if fearfulness would decrease as a result of genetic selection. It is not desirable to breed chickens that are unresponsive to their environment, which can be perceived as unethical due to a loss of naturalness and animal integrity (D’Eath et al., 2010). However, domestication has been a process of selective breeding (Jones and Hocking, 1999) in which a low level of fearfulness was one of the selection criteria, and this could be the next step.

Other possible applications of the APS, once fully operational, are numerous. It is not (yet) intended to be used as an on-farm technology for the farmer, because this would be too costly, without sufficient direct benefit. However, for research it could be used in many ways. It could be used to monitor free-range use to further identify factors that can promote this. If all birds in a group are monitored simultaneously, it can be used to perform social network analyses. It could be used to identify early onset of e.g. disease, lameness, feather pecking, cannibalism, smothering or keel bone damage by monitoring aspects such as individual birds' daily activity patterns. These patterns have been shown to be highly consistent in laying hens (Vögeli et al., 2017), although this may not be true for all individuals (Larsen et al., 2017). A change in individual patterns or a decrease in total distance travelled per day could imply the start of disease (Frost et al., 1997). If the Z coordinates of the system can also be used, it may also be used to monitor use of platforms and perches, or different tiers in aviary systems. Another application could be more fundamental ethological research.

In addition to using the system for chickens it could also be used to track other species. Currently, a system using the same technology is being tested and used at ILVO for sows housed in groups indoors, and it appears that the system also works in an indoor environment containing metal structures.

When using automated tracking technology, it is important that the devices that are attached to the chickens do not disturb their behaviour, production, health and/or longevity (depending on the aim of the research) so that the results gathered with the system are representative for birds without these devices. Therefore, the effects of wearing the tags of the UWB system used in this thesis on slow-growing broiler chickens' behaviour, leg health (gait, FPD, hock dermatitis) and weight gain have been tested (Stadig et al., 2017). No effects on leg health and weight gain in the period the birds wore the tags were observed. Small differences in behaviour occurred in the first week after the birds were fitted with the tags: birds with tags walked less and pecked less at other birds with a tag than birds identified using colour marking. However, these results were not observed anymore after this first week, implying that birds either habituated to the tags quickly, or experienced less discomfort due to a higher BW over time, and thus a smaller BW to tag weight ratio.

8.5 Practical implications

8.5.1 Shelter provision

This thesis demonstrated the positive effects of providing suitable shelter on free-range use by slow-growing broiler chickens. Free-range systems were designed to be better for animal welfare, and free-range eggs and poultry meat are also marketed as such. It is therefore important to meet the needs of the chickens. Current legislation states that range areas should be “mainly covered with vegetation and be provided with protective facilities” in the case of organic production (European Commission, 2008b), and that it should be “mainly covered by vegetation” for free-range production (European Commission, 2008a). It is up to certifiers how this is enforced in practice. For example, the organic certifier in the Netherlands, Skal, encourages plantation in free-range areas (I. de Groot, Skal, personal communication).

From a birds’ point of view, SRCW is very suitable as vegetation on the free-range with as main disadvantage that it is usually harvested every three years, creating a period of relatively little shelter for 4 to 5 months following this harvest. However, it is to be recommended to provide natural instead of artificial shelter, as this and other studies demonstrated greater effects of such shelter on free-range use.

8.5.2 Rearing methods

Dark brooders and environmental enrichment were not successful in reducing fearfulness, increasing exploration behaviour or improving free-range use. Therefore, at this point we cannot yet recommend their use in order to achieve these aims. Nevertheless, they may have other benefits for the chickens, which were not assessed in the current study.

8.5.3 SRCW production

This thesis showed that biomass production of SRCW in a free-range area of broiler chickens resulted in yields comparable to yields in other circumstances. Farmers acknowledged the potential benefits for their chickens. Economic profitability can be achieved, but is often low. SRCW would be more economically interesting under certain conditions such as subsidies, high wood chip prices or price premiums for the poultry products. In addition, a solid biomass market and potential collaborations among poultry farmers or between poultry farmers and SRCW producers could aid in increasing SRCW production. If farmers would have to choose between different plots for SRCW production, it would be best not to use plots with high soil levels of N_{min} because these could increase over time. Also, soils that are more compact or contain a high amount of clay could be less at risk of leaching.

8.6 Future research possibilities

Several weather conditions that were experienced as unfavourable by slow-growing broiler chickens have been identified. Rainfall data were dichotomised (rain/no rain) due to a lack of data points with rain. However, it would still be interesting to study the effect of rain intensity on free-range use, because informal observations revealed that birds were outside during periods with little rain, but not during heavy showers.

The rearing strategies tested in this thesis did not succeed in increasing free-range use. Other rearing or incubation strategies aimed at reducing fearfulness could be tested in future research, because there are indications that this is linked to increased free-range use. Studies on light provision during incubation of the eggs, for example, have shown that light regime is correlated with fear and stress responses in broiler chickens (Archer and Mench, 2014, 2013)

Recent research by Taylor et al. (2017b) has shown there is considerable variability between the ranging patterns of individual broiler chickens. More research on individual free-range use will likely provide more clarity on why chickens range and how they can be stimulated to do so. It could also elucidate the relationships between free-range use and other parameters such as leg health and meat quality. Related to this, it would be valuable to perform more studies with the APS. It could e.g. be tested if it can also be used to differentiate between active and non-active birds, or if it could be used to monitor the performance of different behaviours.

Regarding the effects of free-range access on meat quality, linking it to free-range use may make it possible to also connect it to related parameters such as activity or preference for certain vegetation or ground cover (on which can be foraged). In addition, monitoring what and how much is ingested by the birds that are outside could be important because diet can considerably influence meat quality. Together, these approaches may reveal why studies so far have shown contradicting results, and how free-range access and meat quality are actually related.

This thesis demonstrated that SRCW was successful in promoting free-range use. In this study, the SRCW plantation was uninterrupted, there were no open areas between the trees. It is not known if the absence of open areas is required to obtain good free-range use. Also, the exact density of the vegetation, the amount of sunlight that it blocks, the planting direction, the type and amount of ground cover growing underneath the woody vegetation and many other factors may influence the success of the vegetation in promoting free-range use.

The effects of vegetation type, such as SRCW, and chicken presence on soil parameters remain to be further investigated. Although the present study reported high levels of N and P in the soil of SRCW plots, other studies found that SRCW can remove these nutrients from the soil, and can take up cadmium and possibly other heavy metals as well (Dickinson and Pulford, 2005).

8.7 Conclusions

After completion of this thesis, in which the combination of slow-growing broiler chickens and short rotation coppice was studied, as well as the development of an APS for monitoring chickens' positions, it can be concluded that:

- SRCW and broiler chickens can be combined in order to promote free-range use: birds preferred SRCW over AS, and ranged farther from their house in the former.
- Provision of environmental enrichment or dark brooders early in the birds' life did not affect free-range use later in life.
- Overhangs adjacent to the pop holes were not successful in promoting free-range use.
- Free-range access may be associated with better gait and less hock dermatitis, as well as with changes in meat quality such as a more pronounced yellow colour and more tender and less fibrous meat. Individual free-range use monitoring would possibly elucidate these relationships further.
- Welfare benefits regarding leg health or fearfulness of using SRCW instead of grassland could not be confirmed.
- Combining broiler chickens with SRCW had no negative effects on tree growth and biomass production.
- Close to the chicken houses, combining broilers with SRCW resulted in high levels of N and P in the soil as compared to grassland. Close to the house this is most likely due to presence of chickens, in areas farther from the houses or outside of the chicken range it was due to the SRCW itself, with litter fall leading to return of nutrients to the soil. It remains to be further examined to what extent these high nutrient levels also entail an actual risk of nutrient leaching.
- The APS that was developed showed promise for use in future research. There could be some bias between different vegetation types, with tags in SRCW being detected less often than those in grassland, although this was probably at least partially due to problems with anchors next to the SRCW plots.

Summary

Chickens with free-range access often make limited use of their free-range area; a small part of the flock is usually observed outside at any given time, and those that are outside prefer to stay close to their houses. As a consequence, the claimed welfare benefits of free-range use may not be achieved, and nutrient loads in the soil are very high close to the chicken houses. The low range use may have several reasons, such as fear of a novel environment, adverse weather conditions, or low motivation to explore. This thesis aimed to assess the effects of combining slow-growing free-range broiler chickens with short rotation coppice willows (SRCW). These are fast-growing trees that are used for biomass production. They could provide a good ranging environment to the chickens, and be an extra source of income for the poultry farmer. It was assessed how SRCW and artificial shelter influenced free-range use, leg health, fearfulness and meat quality. In addition, two rearing strategies were tested aimed at improving free-range use. Furthermore, a new system was developed to automatically monitor free-range chickens' position. Finally, the interactions between chickens, SRCW and soil parameters were assessed.

Factors affecting and being affected by free-range use

In Chapters 2, 3 and 4 the relationships between free-range use and shelter types were studied. In Chapter 2, slow-growing broiler chickens (Sasso XL451) were given outdoor access either to an area with grassland and artificial shelters (wooden A-frames; AS) or to an area with SRCW. In all studies in this thesis, birds were given outdoor access approximately between four and ten weeks of age. The groups with access to SRCW had higher mean percentages of birds outside (42.8% vs. 35.1%), and more birds that ranged farther than 5 m from the house (10.6% vs. 4.1% of all birds outside). In Chapters 3 and 4 the birds were given access to both AS and SRCW. This revealed that birds had a strong preference for SRCW, with more birds ranging in this shelter type and with birds going farther from their house. In Chapter 4, an additional shelter type was provided, i.e. overhangs adjacent to the pop holes. It was hypothesised that these would result in a more gradual transition between the indoor and outdoor environment, and therefore in more free-range use. However, no such effect was found, neither were overhangs related to a difference in the behaviours that the birds displayed.

The effects of weather conditions on free-range use were studied in Chapters 2 and 3. If birds had access to either AS or SRCW (Chapter 2), rainfall, increasing solar radiation and increasing wind speed were negatively related with the number of birds outside, and these effects were more pronounced in SRCW. This could indicate that SRCW provides less protection against these weather conditions than the A-frames, but possibly this result was due to more birds being outside in SRCW, so more birds could go inside during adverse weather. If birds had access to both AS and SRCW (Chapter 3), rainfall and decreasing solar radiation were related to finding more birds outside in AS, whereas the opposite was true in SRCW. This suggests that SRCW provides better protection against solar radiation than AS, and that birds chose to seek shelter in the vegetation instead of in their house if they have the opportunity. In this case, increasing wind speed was related to less birds outside in both shelter types. In both chapters, an increasing temperature was related to more birds being outside.

The relationships between free-range access and fearfulness and leg health were studied in Chapter 2. In addition to the birds with access to either AS or SRCW, there were also groups that were kept indoors (IN) for the entire production period. In week 3 (i.e. before outdoor access was provided), birds were subjected to a tonic immobility (TI) test (gives an indication of the level of fearfulness), and this test was repeated in week 10. A longer TI duration in week 3 was associated with more birds farther than 5 m from the house, but not with the mean number of birds outside. TI duration in week 10 was not associated with either of these, but the number of inductions needed was higher in SRCW than in IN groups. These findings suggest that there is a negative relationship between fearfulness and free-range use, but more studies, e.g. on individual birds' data, are needed to confirm this. Gait problems tended to occur more in IN than in AS birds, and hock dermatitis occurred more in IN than in AS, and tended to occur more in IN than in SRCW.

The behaviours of the birds in relationship to the shelter types were studied in Chapters 3 and 4. This revealed that relatively more birds were foraging in AS, but because the total number of birds was always higher in SRCW, the absolute number of birds foraging was also higher in SRCW. Foraging occurred more at >5 m from the house than closer by, possibly due to depletion of vegetation in proximity of the houses. Sitting occurred more close to the houses, and in SRCW, which may be attributed e.g. to a more favourable microclimate or a greater sense of safety due to more cover.

In Chapters 3 and 4, two rearing strategies were tested: providing environmental enrichment and providing access to dark brooders early in the chickens' lives. Both were provided from day 0 until the birds were moved to mobile houses in week 4. The enrichment consisted of hay bales, scattered grain, strings and live mealworms. Dark brooders are warm, dark, secluded areas in the home pen under which the chicks can rest. There are indications that both environmental enrichment and dark brooders have the potential to decrease fearfulness and increase exploration motivations, which could subsequently lead to better free-range use later in life. In the present study, the enrichment and dark brooders had no relevant effect on TI duration or free-range use. The dark brooders only tended to affect the number of birds that jumped in an open field test (higher in non-brooded birds). No effects of the dark brooders on behaviour of the birds at later age could be demonstrated, and only minimal effects of the enrichment on behaviours were found.

In Chapter 5, the effect of free-range use on production and meat quality was assessed. The treatment groups used were the same as in Chapter 2 (IN, AS, SRCW). At slaughter age (d72), IN birds were heavier than AS and SRCW birds, but no differences in feed intake or feed conversion were found, possibly due to unregistered feed intake (vegetation, insects, small vertebrates) by the AS and SRCW birds in the free-range areas. Breast meat of chickens with free-range access was darker and yellower than that of IN chickens. Ultimate pH was lower and drip loss higher in IN versus AS chickens. The percentage of polyunsaturated fatty acids was higher in AS than in IN meat. A blinded taste panel judged breast meat of SRCW chickens to be more tender and less fibrous compared to that of AS and IN chickens, and juicier compared to the IN chickens.

Automated positioning system

Chapter 6 describes the performance of a newly developed automated positioning system to monitor free-range chickens’ position. This Ultra-Wideband (UWB) system consists of active tags (attached to the chickens) that send signals to anchors positioned at fixed locations in the field; the tags’ position can be calculated using the time of arrival of its signal, if this is registered by at least three anchors. Its accuracy and registration success, as well as which factors may affect its performance, were assessed. The effects of vegetation type, precipitation, tags being mounted on a chicken, tag height, angle and orientation, coverage by A-frames or mobile chicken houses, and proximity of other tags on accuracy of the registered positions (distance between the registered and the true position of the tag) and on registration success (percentage of registrations where a position could be calculated) were assessed.

Overall, the median error was 0.29 m, and the mean percentage of successfully registered positions was 68%. None of the variables had a clear effect on the accuracy of the positions. Errors were generally larger in certain areas of the experimental field, which may be due to the asymmetrical setup of the anchors. The percentage of successful registrations was negatively affected by shelter type, with lower percentages in dense vegetation (short rotation coppice willows) than on grassland, possibly due to malfunctioning of two anchors close to the SRCW plots. Rain and placing the tags underneath a wooden A-frame, but not placing them in a mobile house, resulted in a lower percentage of successful registrations. The tag being mounted on a chicken, height and angle of the tag and proximity of other tags had no negative effect on the percentage of successful registrations. Placing more (functioning) anchors may contribute to better accuracy and registration success. Alternatively, the bias resulting from the variables that had a negative effect on registration success should be corrected for when using the system in its current setup. Overall, this system shows great promise to be used for monitoring chickens' free-range use.

Interactions between chickens, SRCW and soil parameters

In Chapter 7, the interactions between slow-growing broilers, SRCW and soil parameters were studied. The experimental field was split up into four quadrants: two were sown with a grass/clover mixture, two were planted with SRCW (three clones, i.e. Tora, Tordis and Klara) and clover as undergrowth. SRCW was harvested 1 and 4 years after establishment. Chickens were present on the field during parts of each year (see Chapters 2, 3 and 4), and parts of the field were kept chicken-free as a control. Free-range use, SRCW growth and soil parameters were monitored on a regular basis over a 4-year period.

No effects of chicken presence on SRCW growth were observed. Total mineral N (N_{min}) was affected by vegetation type x location x depth; it was generally higher in SRCW than in grassland, in areas close to the chicken houses, and in more superficial soil layers. This could be due to return of N through leaf fall, as opposed to grass which is mown and removed. SRCW was also harvested eventually, but the amount of N removed through this process was lower than that removed by mowing the grassland. In addition, higher N_{min} levels could be due to the higher chicken density in SRCW (more N deposition through faeces), to NH_3 being captured from the air by the trees, to the strong clover development under SRCW (which can fix atmospheric N), and to the lower N requirement of SRCW compared to grassland. N_{min} did not appear to accumulate in the soil over the years, but close to the chicken houses there were indications for nitrate leaching to deeper soil layers and possibly to groundwater. K and P-$CaCl_2$ were higher close to the chicken houses, probably due to high concentrations of these nutrients in chicken faeces. No increase in soil organic C was observed over the four-year experimental period, and no differences were found between SRCW and grassland. This could be due to the short time period that SRCW was present. In conclusion, SRCW was preferred by the chickens, but the possible leaching of nitrate to ground water close to the houses and possible remediating strategies for these need to be studied further.

Conclusions

From this thesis it can be concluded that SRCW and broiler chickens can be combined in order to promote free-range use: birds preferred SRCW over AS, and ranged further from their house in the former, without having an effect on SRCW production. Overhangs adjacent to the pop holes were not successful in promoting free-range use. The provision of environmental enrichment or dark brooders early in the birds' life did not affect free-range use later in life. Free-range access may be associated with better gait and less hock dermatitis, as well as with changes in meat quality such as a more pronounced yellow colour and more tender and less fibrous meat. Individual free-range use monitoring would possibly elucidate these relationships further. The automated positioning system that was developed showed promise for use in future research. There could be some bias between different vegetation types, with tags in SRCW being detected less often than those in grassland, although this was probably at least partially due to problems with anchors next to the SRCW plots. Combining broiler chickens with SRCW resulted in high levels of N and P in the soil, especially close to the chicken houses. This can result in leaching of these nutrients to groundwater, and calls for further research.

Samenvatting

Kippen met toegang tot een uitloop maken vaak maar weinig gebruik van deze uitloop; vaak bevindt zich slechts een klein deel van de dieren buiten, en deze blijven vaak dicht bij de stallen. Daardoor worden de welzijnsvoordelen van vrije uitloop mogelijk niet gerealiseerd, en de hoge concentratie kippen dicht bij de stallen vormt een risico op puntvervuiling. Er zijn verschillende redenen voor het beperkte uitloopgebruik, zoals angst voor een nieuwe omgeving, ongunstige weersomstandigheden, of weinig motivatie om te exploreren. Deze thesis had als doel om de effecten te bestuderen van het combineren van enerzijds langzaamgroeiende vleeskippen met vrije uitloop en anderzijds de productie van korte-omloophout (KOH). Dit zijn snelgroeiende bomen, in dit geval wilgen, die worden geteeld voor hun biomassaproductie waarmee vaak energie wordt opgewekt. KOH kan een goede beschutting bieden aan de kippen in de uitloop, en het kan een extra bron van inkomsten zijn voor de pluimveehouder. De effecten van KOH en kunstmatige beschutting op uitloopgebruik, pootgezondheid, angstigheid en vleeskwaliteit werden onderzocht. Daarnaast werden twee opfokstrategieën getest, met als doel het verbeteren van het uitloopgebruik. Daarnaast werd een nieuw systeem ontwikkeld om de locatie van de kippen in de uitloop automatisch te kunnen registreren. Tenslotte werden de interacties tussen de kippen, KOH en bodemparameters onderzocht.

Welke factoren beïnvloeden en worden beïnvloed door vrije-uitloopgebruik?

In hoofdstukken 2, 3 en 4 werden de relaties tussen uitloopgebruik en beschuttingstype onderzocht. In hoofdstuk 2 kregen langzaamgroeiende vleeskippen (Sasso XL451) toegang tot ofwel een uitloop met grasland en kunstmatige beschutting (houten A-panelen; KB) ofwel een uitloop met korte-omloophout. In alle onderzoeken van deze thesis kregen de kippen toegang tot de uitloop van 4 tot 10 weken leeftijd. Het uitloopgebruik van de groepen met toegang tot KOH was hoger (42,8%) dan dat van groepen met toegang tot KB (35,1%). Ook bevond een groter deel van de kippen die buiten waren zich verder dan 5 m van de stal (10,6% vs. 4,1%). In hoofdstukken 3 en 4 hadden de kippen toegang tot zowel KB als KOH. Hieruit bleek dat de kippen een sterke voorkeur hadden voor KOH: er gingen meer kippen naar buiten en de dieren bevonden zich verder van de stallen. In hoofdstuk 4 werd ook getest of het aanbieden van afdakjes aan de stalopeningen zou resulteren in meer uitloopgebruik, omdat deze zorgen voor een meer geleidelijke overgang van binnen naar buiten. Dit was niet het geval, en ook werd geen effect van de afdakjes gevonden op de gedragingen van de kippen in de uitloop.

De effecten van weersomstandigheden op uitloopgebruik werden onderzocht in hoofdstukken 2 en 3. Als kippen toegang hadden tot ofwel KB ofwel KOH (hoofdstuk 2), hadden regen, toenemende zonnestraling en toenemende windsnelheid een negatieve relatie met het aantal dieren buiten, en deze effecten waren sterker in KOH. Dit kan betekenen dat KOH minder goede beschutting bood tegen deze weersomstandigheden dan KB, maar de bevindingen kunnen ook komen doordat er meer kippen buiten waren bij KOH dan bij KB, waardoor er ook meer dieren naar binnen konden gaan bij slecht weer. Wanneer de dieren toegang hadden tot zowel KB als KOH (hoofdstuk 3), waren regen en afnemende zonnestraling geassocieerd met meer kippen buiten bij KB, en omgekeerd voor KOH. Dit suggereert dat KOH een betere bescherming biedt tegen zonnestraling dan KB, en dat de kippen in dat geval voor kiezen om beschutting te zoeken in de vegetatie in plaats van in de stal. Een toenemende windsnelheid was wederom geassocieerd met minder kippen buiten (in beide beschuttingstypes). In beide hoofdstukken was er een positieve relatie tussen omgevingstemperatuur en het aantal kippen buiten.

De relaties tussen uitloopgebruik en angstigheid en pootgezondheid werden onderzocht in hoofdstuk 2. Naast de groepen kippen die toegang hadden tot een uitloop met KB of KOH, werd ook een aantal groepen de volledige productieperiode binnen gehouden (BIN). In week 3 (d.w.z. voordat de dieren toegang tot de uitloop kregen) ondergingen de dieren een tonische immobiliteit (TI) test (indicatief voor de mate van angstigheid), welke werd herhaald in week 10. Een langere TI-duur in week 3 was geassocieerd met meer kippen die zich verder dan 5 m van de stal bevonden, maar niet met het gemiddeld aantal kippen dat buiten was. De TI-duur in week 10 was niet gerelateerd met één van deze metingen, maar het aantal inducties dat nodig was om tot TI te komen was hoger in groepen met KOH dan BIN. Deze resultaten zijn een indicatie voor een negatieve relatie tussen angstigheid en uitloopgebruik, maar er zijn meer studies nodig om dit te bevestigen, bijvoorbeeld op data van individuele dieren in plaats van op groepsniveau. Er was een trend dat kreupelheid meer voorkwam bij BIN dan bij KB-kippen. Hakdermatitis kwam meer voor bij BIN dan bij KB-kippen, en er was een trend dat dit meer voorkwam bij BIN dan bij KOH-kippen.

De gedragingen van de kippen in relatie tot beschuttingstype werden onderzocht in hoofdstukken 3 en 4. Hieruit bleek dat relatief meer dieren foerageerden in KB, maar omdat het totaal aantal kippen gemiddeld hoger was in KOH was het absolute aantal dieren dat foerageerde in KOH ook hoger. Foerageren kwam meer voor of >5 m van de stal dan dichterbij, mogelijk door depletie van de vegetatie dicht bij de stallen. Zitten kwam meer voor dicht bij de stallen en in KOH, wat mogelijk kan komen door een gunstiger microklimaat of een groter gevoel van veiligheid door meer beschutting dan bij KB.

In hoofdstukken 3 en 4 werden twee opfokstrategieën getest, namelijk het aanbieden van omgevingsverrijking (hooibalen, meelwormen, verspreid graan en touw) of toegang tot ‘dark brooders’ tijdens het vroege leven van de kuikens. Dark brooders zijn warme, donkere ruimtes in het hok waar rustende kuikens zich kunnen afzonderen van de actieve dieren. Beide werden voorzien tussen dag 0 en dag 25, waarna de dieren werden verhuisd naar mobiele stallen om toegang tot uitloop te kunnen krijgen. Eerder onderzoek wees uit dat zowel omgevingsverrijking als dark brooders potentieel hebben om angstigheid te verlagen, en de motivatie om te exploreren te verhogen, wat uiteindelijk zou kunnen leiden tot een beter uitloopgebruik. In de huidige studie hadden de omgevingsverrijking en dark brooders geen relevant effect op de TI-duur of uitloopgebruik. In een open field test werd slechts één verschil gevonden dat er mogelijk op wijst dat de dieren met dark brooders mogelijk angstiger zijn. Er konden geen effecten worden aangetoond van dark brooders op het gedrag van de kippen op latere leeftijd, en omgevingsverrijking resulteerde slechts in kleine verschillen.

In hoofdstuk 5 werd het effect van uitloopgebruik op productieparameters en vleeskwaliteit onderzocht. De behandelingen waren gelijk aan die in hoofdstuk 2 (BIN, KB, KOH). BIN-kippen waren op slachtleeftijd (d72) zwaarder dan KB- en KOH-kippen, maar er konden geen verschillen in voederopname of voederconversie worden aangetoond. Dit kan komen doordat de voederopname in de uitloop (vegetatie, insecten, kleine vertebraten) door de KB- en KOH-kippen niet geregistreerd werd, waardoor hun werkelijke voederopname en –conversie hoger liggen. Het borstvlees van kippen met uitlooptoegang was donkerder en geler dan dat van BIN-kippen. De pH was lager en druipverlies hoger in BIN- dan in KB-kippen. Het percentage meervoudig onverzadigde vetzuren was hoger in vlees van KB- dan van BIN-kippen. Een blinde smaaktest wees uit dat het vlees van kippen met toegang tot KOH malser en minder vezelig was dan dat van BIN- en KB-kippen, en sappiger dan dat van BIN-kippen.

Automatisch positiebepalingssysteem

In hoofdstuk 6 wordt de prestatie van een nieuw-ontwikkeld automatisch positiebepalingssysteem (APS) getest, dat gebruikt kan worden om de locatie van kippen met een uitloop te registreren. Dit Ultra-Wideband (UWB) systeem bestaat uit actieve tags (die worden bevestigd aan de kippen) die een signaal uitzenden dat ontvangen wordt door ankers welke op verschillende, vaste plaatsen op het veld staan. De locatie van de tag kan worden berekend op basis van de time-of-arrival van het signaal, als dit door minimaal drie ankers is ontvangen. De accuraatheid (d.w.z. het verschil tussen de gemeten en de echte locatie van de tag) en het registratiesucces (d.w.z. het percentage van de registraties waarbij een locatie berekend kon worden), alsook factoren die dit mogelijk konden beïnvloeden, werden getest. De effecten van vegetatie, neerslag, het plaatsen van de tag op een kip, het plaatsen van de tag onder een A-paneel of in een mobiele stal, nabijheid van andere tags, en de hoogte, oriëntatie en hoek van de tags werden onderzocht.

De mediaan van de fout van alle metingen was 0,29 m, en het gemiddeld percentage succesvolle registraties was 68%. Geen van de bovengenoemde geteste factoren had een duidelijk effect op de accuraatheid van de geregistreerde locaties. Wel waren de fouten gemiddeld groter in bepaalde gebieden van het proefveld, wat te wijten kan zijn aan een asymmetrische opstelling van de ankers. Het registratiesuccess werd negatief beïnvloed door beschuttingstype: in het KOH was dit percentage lager dan op grasland. Dit komt mogelijk door het niet goed functioneren van twee ankers die aan de KOH-percelen grensden. Neerslag en het plaatsen van de tags onder de A-panelen (maar niet in de mobiele stallen) zorgden voor een lager registratiesucces. Het plaatsen van de tag op een kip, hoogte en hoek van de tag, en nabijheid van andere tags hadden geen negatief effect op het registratiesucces. Het plaatsen van meer (goed functionerende) ankers kan bijdragen aan een betere accuraatheid en registratiesucces. Dit systeem lijkt veelbelovend te zijn voor het opvolgen van uitloopgebruik in de toekomst.

Interacties tussen kippen, KOH en bodemparameters

In hoofdstuk 7 werden de interacties tussen langzaamgroeiende vleeskippen, KOH en bodemparameters bestudeerd. Het proefveld was opgedeeld in vier kwadranten: twee werden ingezaaid met een grasklavermengsel, twee werden beplant met KOH (drie klonen: Tora, Tordis en Klara) met klaver als ondergroei. KOH werd 1 en 4 jaar na het aanplanten geoogst. Ieder jaar waren gedurende verschillende periodes kippen op het veld aanwezig (zie hoofdstukken 2, 3 en 4). Ook werden delen van het veld ‘kip-vrij’ gehouden als controle. Uitloopgebruik, groei van het KOH en bodemparameters werden regelmatig opgevolgd gedurende vier jaar.

De aanwezigheid van de kippen had geen effect op de groei van het KOH. Totale minerale stikstof (N_{min}) werd beïnvloed door een interactie tussen vegetatie, locatie en diepte; over het algemeen was N_{min} hoger in KOH dan in grasland, in gebieden dicht bij de kippenstallen, en in de meer oppervlakkige bodemlagen. De hogere gehaltes in KOH kunnen komen door recyclage van N door bladval, terwijl gras regelmatig werd gemaaid en afgevoerd. KOH werd uiteindelijk ook geoogst en afgevoerd, maar hierbij werd minder N van het veld verwijderd dan bij het maaien. Daarnaast kunnen de hogere gehaltes N_{min} te wijten zijn aan de hogere kippendichtheid in KOH (meer N-depositie via de faeces), aan het afvangen van ammoniak uit de lucht door de bomen, aan de sterke ontwikkeling van klaver onder het KOH (dit kan stikstof uit de lucht fixeren), en aan een lagere N-behoefte van KOH vergeleken met grasland. N_{min} leek niet te accumuleren over de jaren heen, maar dicht bij de stallen waren wel indicaties voor uitspoeling van nitraat naar diepere bodemlagen en mogelijk naar het grondwater. Kalium en fosfor (P) waren hoger dicht bij de kippenstallen, waarschijnlijk door hoge concentraties van deze nutriënten in de kippenfaeces. Er werd geen toename van organische koolstof gemeten in deze vier jaar, en ook was er geen verschil tussen KOH en grasland; mogelijk was de onderzoeksperiode hier te kort voor. Er kan geconcludeerd worden dat kippen een voorkeur hadden voor KOH, maar de mogelijke uitspoeling van nitraat en mogelijke oplossingen hiervoor moeten verder onderzocht worden.

Conclusies

Uit deze thesis kan geconcludeerd worden dat de combinatie van vleeskippen en KOH succesvol is wat betreft het verbeteren van het uitloopgebruik van de kippen. De dieren hadden een duidelijke voorkeur voor KOH en kwamen bij dit beschuttingstype ook verder van de stallen, zonder dat een effect op KOH-productie werd gemeten. Afdakjes aan de stalopeningen hadden geen effect op uitloopgebruik. Het aanbieden van omgevingsverrijking en dark brooders tijdens het vroege leven van de kuikens had geen effect op uitloopgebruik later in hun leven. Uitloopgebruik is geassocieerd met minder kreupelheid en hakdermatitis, alsook met veranderingen in de vleeskwaliteit zoals geler, malser en minder vezelig vlees. Het monitoren van individueel uitloopgebruik zou meer informatie kunnen geven over deze relaties. Het automatische positiebepalingssysteem dat werd ontwikkeld is veelbelovend. Wel was er momenteel nog een bias gerelateerd aan vegetatietype, waarbij tags in het KOH minder goed gedetecteerd werden dan die op grasland. Dit was waarschijnlijk (deels) te wijten aan problemen met de ankers die aan de KOH-percelen grensden. Het combineren van vleeskippen met KOH resulteerde in hoge gehaltes van N en P in de bodem, voornamelijk dicht bij de kippenstallen. Dit kan resulteren in een risico op uitspoeling van deze nutriënten, en moet verder onderzocht worden.

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Appendix A

Model 1: $y = \beta_0 + distance * \beta_{distance} + clone * \beta_{clone} + distance * clone * \beta_{distance*clone} + \beta_{quadrant} + \varepsilon$

Where:
y = diameter, height or dry weight of the trees (measured on individual trees)
Distance = 6, 12, 18, 24, 30, or 31-36 m from the house or 'in chicken-free area'
Clone = Tora, Tordis or Klara

Model 2: $y = \beta_0 + location * \beta_{location} + clone * \beta_{clone} + location * clone * \beta_{location*clone} + \beta_{quadrant} + \varepsilon$

Where:
y = dry weight of the trees (based on total harvest)
Location = inside or outside chicken range
Clone = Tora, Tordis or Klara

Model 3: $N_{min} = \beta_0 + location * \beta_{location} + vegetation * \beta_{vegetation} + depth * \beta_{depth} + location * vegetation * \beta_{location*vegetation} + location * depth * \beta_{location*depth} + vegetation * depth * \beta_{vegetation*depth} + location * vegetation * depth * \beta_{location*vegetation*depth} + \beta_{quadrant} + \varepsilon$

Where:
Location = 1 m from chicken house, 5 m from chicken house, >10 m from chicken house, or in chicken-free area
Vegetation = grass or SRCW
Depth = 0-30 cm, 30-60 cm or 60-90 cm

Model 4: $y = \beta_0 + location * \beta_{location} + vegetation * \beta_{vegetation} + location * vegetation * \beta_{location*vegetation} + \beta_{quadrant} + \varepsilon$

Where:
y = NO_3-N and NH_4-N in the 0-90 cm layer
Location = 1 m from chicken house, 5 m from chicken house, >10 m from chicken house, or in chicken-free area
Vegetation = grass or SRCW

Model 5: $y = \beta_0 + location * \beta_{location} + \ vegetation * \beta_{vegetation} + depth * \beta_{depth} + \ location * depth * \ \beta_{location*depth} + vegetation * depth * \ \beta_{vegetation*depth} + \beta_{quadrant} + \varepsilon$

Where:
y = NO_3-N and NH_4-N
Location = 1 m from chicken house, 5 m from chicken house, >10 m from chicken house, or in chicken-free area
Vegetation = grass or SRCW
Depth = 0-30 cm, 30-60 cm or 60-90 cm

Model 6: $y = \beta_0 + date * \beta_{date} + \ location * \beta_{location} + \ vegetation * \beta_{vegetation} + date * \ location * \beta_{date*location} + date * \ vegetation * \beta_{date*vegetation} + vegetation * location * \beta_{vegetation*location} + \beta_{quadrant} + \beta_{locationID} + \varepsilon$

Where:
y = NO_3-N, NH_4-N or total N_{min}
Date = October 2013, October 2014, October 2015, October 2016, or February 2017
Location = 1 m from chicken house, 5 m from chicken house, >10 m from chicken house, or in chicken-free area
Vegetation = grass or SRCW
Depth = 0-30 cm, 30-60 cm or 60-90 cm

Model 7: $y = \beta_0 + date * \beta_{date} + \ location * \beta_{location} + \ vegetation * \beta_{vegetation} + depth * \beta_{depth} + \ date * location * \beta_{date*location} + date * \ vegetation * \beta_{date*vegetation} + date * depth * \ \beta_{date*depth} + location * vegetation * \beta_{location*vegetation} + location * depth * +\beta_{location*depth} + vegetation * depth * \beta_{vegetation*depth} + date * location * vegetation * \beta_{date*location*vegetation} + date * location * depth * \beta_{date*location*depth} + date * vegetation * depth * \beta_{date*vegetation*depth} + location * vegetation * depth * \beta_{location*vegetation*depth} + date * location * vegetation * depth * \beta_{date*location*vegetation*depth} + \beta_{quadrant} + \beta_{locationID} + \varepsilon$

Where:
y = N_{min} (0-90 cm) in grassland at 1 m from the house, in grassland at >10 m from the house, in SRCW at 1 m from the house, or in SRCW at >10 m from the house
Date = October 2016 or February 2017
Location = 1 m from chicken house or >10 m from chicken house
Vegetation = grass or SRCW
Depth = 0-30 cm, 30-60 cm or 60-90 cm

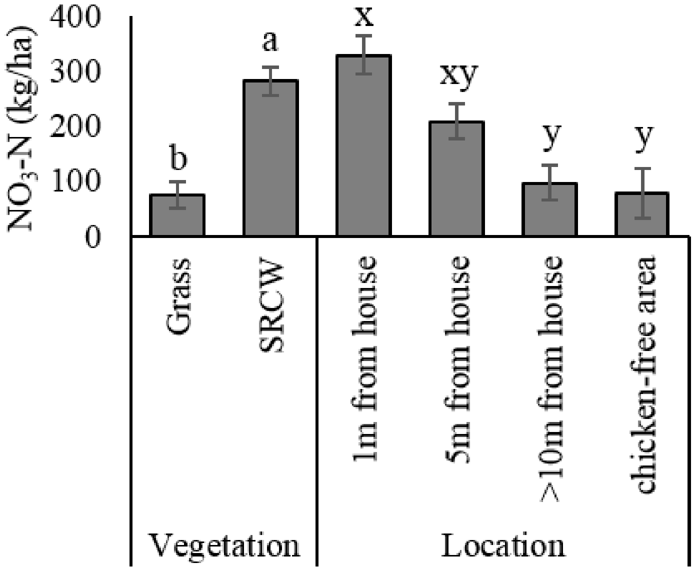
Model 8: $y = \beta_0 + vegetation * \beta_{vegetation} + \ location * \beta_{location} + vegetation * location * \beta_{vegetation*location} + \beta_{quadrant} + \ \varepsilon$

Where:
y = P-$CaCl_2$, TOC, pH, K, Mg, Mn, Fe, P or total N in the top layer (0-10 cm), or TOC in the 0-30 cm layer
Location = 1 m from chicken house, 5 m from chicken house, >10 m from chicken house, or in chicken-free area
Vegetation = grass or SRCW

Model 9: $y = \beta_0 + clone * \beta_{clone} + \ \beta_{quadrant} + \ \varepsilon$

Where:
y = N, P or C content in the leaves in December 2016 or N or P content in the wood at harvest in 2017
Clone = Tora, Tordis or Klara

Appendix B



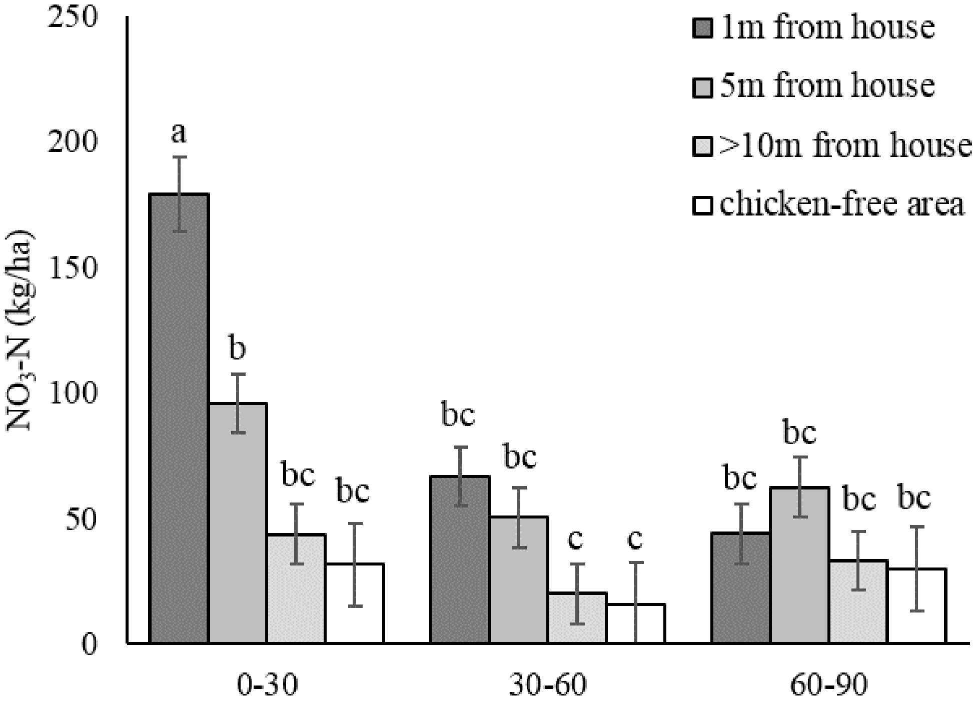
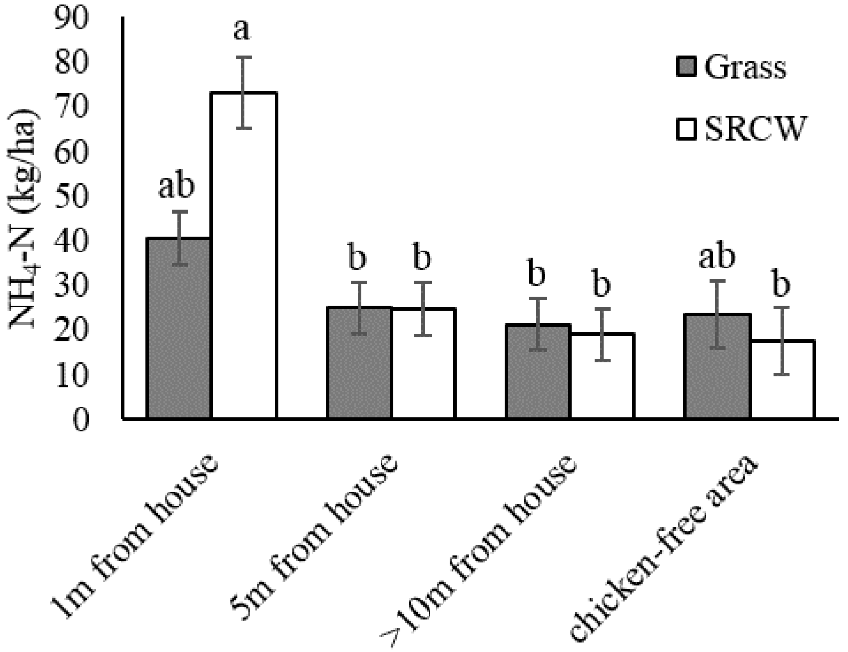


Figure B.1 Above: NO_3-N (up to 90 cm depth) per vegetation type and location. Bars without a common superscript differ significantly within vegetation (a, b) or location (x, y). Below: NO_3-N per soil layer (0-30, 30-60 and 60-90 cm) and location. Bars without a common superscript differ significantly ($P < 0.05$).



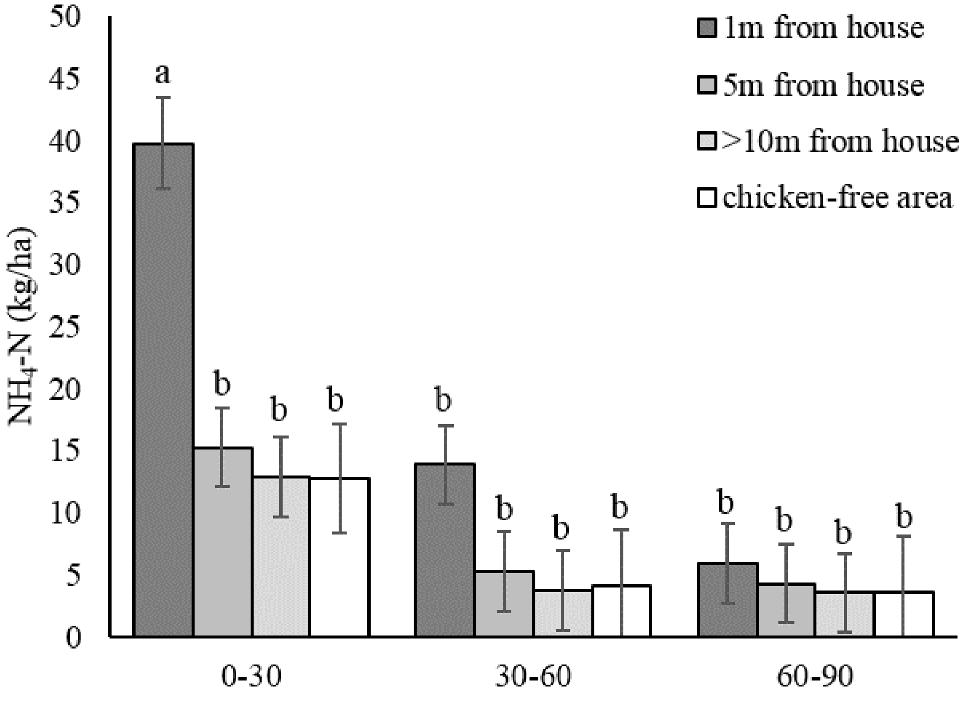


Figure B.2 Above: NH_4-N (total of all layers) per vegetation type and location. Bars without a common superscript differ significantly. Below: NH_4-N per soil layer (0-30, 30-60 and 60-90 cm) and location. Bars without a common superscript differ significantly ($P < 0.05$).

Curriculum Vitae

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Education & work experience

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2010 – 2012	Master in Animal Sciences - Wageningen University, Wageningen, the Netherlands. Specialisation: Animal Health and Behaviour
2005 – 2010	Bachelor in Veterinary Medicine - Utrecht University, Utrecht, the Netherlands

Publications

Papers in international, peer-reviewed scientific journals

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